ANNUAL REPORT
OF
PROGRAM ACTIVITIES

DIVISION OF BIOLOGICS STANDARDS

FISCAL YEAR 1971

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

U.S. DIVISION OF BIOLOGICS STANDARDS

Fiscal Year 1971

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July 1, 1970 through June 30, 1971

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DIVISION OF BIOLOGICS STANDARDS

SUMMARY OF THE DIRECTOR

INTRODUCTION

The mission of the Division is the exercise of controls on the safety, purity and potency of biological products through a system of licensing of manufacturing establishments and of the products, as well as the conduct of research related to these responsibilities in order that decisions made and actions taken can be based on sound information.

The Division has the statutory task of making recommendations for standards for the continued safety, purity and potency for all biological products such as vaccines, serums, human blood and its derivatives, and like products. These include written standards, eventually promulgated as regulations, some of which apply to individual products and some to products in general, and physical standards, that is, preparations whose properties and qualities have been extensively characterized and which are issued to manufacturers and other research laboratories concerned with the standardization of biological products for evaluation with their own products, particularly with respect to potency or strength.

The acquisition of the information needed to develop written standards and the laboratory investigations leading to the development and selection of suitable physical standards are extensive in character and often time-consuming. This is particularly true of standards for the tissue-culture-prepared virus vaccines such as poliovirus, measles, mumps and rubella, which have been developed in recent years.

When a new vaccine against a particular disease appears achievable, it devolves upon the Division to develop standards that will be ready at approximately the time that the product is brought to the final stages of manufacture. This requires considerable research involving most of the component laboratories of the Division, as well as the testing of samples of both experimental and final products. It is necessary during this period to maintain close contact with the manufacturer and with scientists working in the field so that problems related to standards can be resolved during the developmental phases. In addition, any clinical investigations conducted by the manufacturers or by investigators studying the products under a variety of conditions come under review of the Division.

When a manufacturer decides to market a product which he has developed, he submits a license application to the Division, together with samples of the product and results of all tests. Division scientists then review the application and examine the product in the laboratory to ascertain the validity of the manufacturer's test results. If the tests fulfill the requirements and it can be shown that applicable standards are met, a product license is recommended for approval. If this is the first product subject to license by the manufacturer, his facilities are also inspected, and if established

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standards are met, he is simultaneously recommended for an establishment license. In the case of a manufacturer already licensed for other biological products, production facilities may be inspected as deemed necessary according to the nature of the pending product. The establishment is inspected annually to verify that proper procedures are being followed in manufacturing and testing. Samples and the results of tests performed on each batch or lot of biological products proposed for marketing must be submitted to the Division by the manufacturer prior to distribution, as determined by the Director. The Division checks the test results and runs similar tests to ensure the quality of the products. Only then is a release which permits marketing issued to the manufacturer.

Once a product has come into use, its safety and potency must be monitored. The knowledge and skill required to do this also provides a sound basis of knowledge and experience for consideration of problems associated with newer products as they are developed.

Brief reviews of the control activities, and of licensing inspections, investigations* and legal actions are included in this summary. It is noteworthy, however, that except for the staff involved in the section on licensing and investigations, the scientific personnel of the Division's laboratories, according to their capabilities, participate in inspections and control tests as well as in the research which comprises the main substance of this report.

Continued research on both old and new products is necessary, not only for obtaining information leading to development of improved standards, but also for the development of expertise and scientific competence on the part of the staff. In many respects, this is a more compelling inducement towards compliance than recourse to regulation-based actions.

Because of the Division's concern with products as such, and because of the involvement of the research staff in the regulatory function, a great deal of attention is directed to "applied" research problems. However, there is probably no element within the NIH where there are more examples of the difficulty of using the words "applied" and "basic" truly to define research endeavor.

The scientific staff continually studies new methods and new approaches to the problems associated with biological products because significance of new knowledge in relation to these products is essential to its function. Of continuing major concern are the viral vaccines, some of which, like vaccinia and rabies, are among the first licensed products, while newer ones are continually being developed. The Division must attempt to evaluate the significance of the demonstrated potential of some viruses to produce tumors in animals under special circumstances; it must also be concerned with the problems of covert viruses which may contaminate cell cultures, and other systems used for the propagation of viruses. This requires that older products be reexamined in terms of contemporary scientific advances from time to time.

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* The term "investigation" used here refers to investigations of possible violation of the law and not to scientific or clinical investigations.

The development of new respiratory viral vaccines, and the continual assessment of older products such as inactivated influenza vaccine, require the Division to develop knowledge concerning antibodies involved in serum in comparison with those in respiratory tract secretions, as well as information on the sites of production and on the transport of these antiviral substances across cell membranes. This is particularly true of inactivated vaccines and is considered to be responsible for the less than optimal potency of influenza vaccine, for example. Analogous problems exist in regard to the factors possibly related to hypersensitivity which have apparently contributed to severe illnesses resulting from natural infection following artificial immunization with some experimental inactivated respiratory vaccines, produced from such agents as respiratory syncytial virus and *Mycoplasma*. These are also related to cellular aspects of immunity, hypersensitivity, and allergy, all of which are problems attracting the attention of DBS scientists, as well as scientists working in the fields of immunology, microbiology and epidemiology.

The study of blood proteins also extends beyond their relation to infectious diseases because of the Division's concern with blood and blood products. Study continues on the purification of various plasma components involved in the clotting and fibrinolytic systems.

Recent research on cholera indicates the likelihood that a toxin, or toxins, produced by the vibrio may serve as the basis for the production of new immunogenic agents. DBS staff members are involved in these cholera investigations.

At the same time, the Division is still confronted with such continuing problems as improvement of pertussis and possibly of tuberculosis vaccines.

New techniques of administration of vaccines and other biological products are also studies since these require careful appraisal. In addition, proposals for the use of various oil adjuvants in immunization and in allergy hyposensitization have prompted the Division to develop an extensive series of contract supported research projects to help resolve the issue of the safety of these materials.

The scientific pursuits of the DBS staff represent responses to solve problems related to its mission. To some extent, the staff must initiate investigations because of proposals for new products being developed by other organizations. To some extent, the control functions lead the staff to recognize gaps in existing knowledge of various biological products and their effects. Also, DBS scientists, like their colleagues in other organizations, are motivated by adding to the body of knowledge in their fields.

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SCOPE OF THE CONTROL-TESTING AND LABELING PROGRAMS

The control-testing program operates as follows: Manufacturers of a licensed product submit to the Division samples from each lot of the product, together with protocols containing the results of tests performed on that lot. Some 262 different biological products are currently licensed involving the issuance of 1,246 individual product licenses.

Each protocol is reviewed by the Division to determine if the manufacturer's test results show conformance to established standards. In addition, some or all of the safety, purity and potency tests required on a particular product may be performed by the Division *as a check on the testing performed by the manufacturer*. If the manufacturer's test results and the results of tests performed by the Division are satisfactory, an official release for that lot is sent to the manufacturer. Only then may the manufacturer distribute the product lot for sale under license.

Two tables are included here to summarize the Division-wide control effort. During the 12-month period covered by this report, 5,515 lots of biological products were released, 84 lots were rejected for a variety of reasons, and 74 lots were voluntarily withdrawn by the manufacturer. Table 1 reflects the number of lots released by categories of products.

A protocol showing the manufacturer's results may consist of considerable information requiring up to 200, and, in many cases more, pages, for example, on a lot of Poliovirus Vaccine, Live, Oral, or data on only a page or so for a lot of blood grouping serum. All protocols are recorded and reviewed by members of the Protocol Review and Product Release Section. The protocols are then forwarded to other laboratories within the Division for review, according to their particular expertise. The nature of the product determines the laboratory(s) consulted. For example, a protocol for Measles Virus Vaccine, Live, Attenuated may be reviewed by personnel in six of the Division's laboratories.

Some of the Division personnel are involved in the control program on a full-time basis while many others are involved intermittently as needed according to the subject involved. As the testing of products becomes more specialized, a larger number of the research scientists become involved in control activities.

Table 2 shows the total number of control tests performed by Division personnel during the last year. This gives no measure of the total effort involved. Tests requiring 15 minutes or less are given the same weight as those taking a month or more to complete in this type of compilation. Continual attention is given to test development, and progress in these endeavors is to be found in the project reports of the different laboratories. It is in this area, especially, that staff of the Laboratory of Control Activities work in collaboration with other Division scientists.

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The control activities of the Division also require the maintenance of a large number of different standards and reference materials. These are prepared within the Division or obtained by contract. They are all evaluated and standardized in the Laboratories of DBS and distributed as needed. During the 12-month period covered by this report, 19,471 individual preparations were issued, a marked increase over the 9,105 preparations issued during FY70. This upsurge is not understood and is being studied. It probably represents increased concern with microbiological standardization, both national and international.

The Protocol Review and Product Release Section maintains a file of complaints and reactions to biological products as received by the Division. Included in the file are copies of complaint correspondence and information on any follow-up actions as required. Also retained in the office is a record of lots of biological products recalled from distribution and use either by the manufacturer or as the result of a request by the Division.

The Label Review Section, Office of the Director, reviews and coordinates the review of all labeling used for biologic products. Such labeling includes container and package labels and package circulars used in both domestic and foreign commerce. Each label is carefully reviewed to determine whether compliance has been achieved with Sections 73.600 through 73.605 of the PHS Regulations as well as applicable Regulations issued by the Food and Drug Administration.

The majority of the labels reviewed were for blood, blood products and blood diagnostic reagent substances. The primary review of these labels was performed in the Laboratory of Blood and Blood Products by personnel with special technical training in this field. Similarly, primary review of labels on certain bacterial and viral products was performed in the appropriate laboratory by personnel possessing specialized training in these respective disciplines. All such labels undergo an additional review in the Label Review Section.

During the period covered by this report, over 2,000 pieces of labeling were reviewed and commented upon before being returned to the manufacturer. This represents about a 100% increase over 1968 and a 10% increase over 1969.

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TABLE 1

Product Group	Lots Released	Lots Rejected	Lots Withdrawn	Totals
Antitoxins	8	0	0	8
Therapeutic Serums	8	0	0	8
Blood and Blood Products	1857	3	12	1872
Bacterial Vaccines	106	0	3	109
Toxoids and Toxins	58	0	0	58
Multiple Antigen Preparations	119	2	6	127
Viral and Rickettsial Vaccines	1405	26	2	1433
Diagnostic Substances for Dermal Test	129	0	0	129
Diagnostic Substances for Laboratory Tests	1450	53	51	1554
Miscellaneous	217	0	0	217
Totals	5,357	84	74	5,515

TABLE 2

TOTAL CONTROL TESTS PERFORMED IN REPORTING PERIOD

Type of Test		Number of Tests
Potency	In Vivo	1,541
	In Vitro	9,422
Safety	General	963
	Specific	17,762
Purity		13,057
Total Control Tests		42,745*

* The number of tests performed in FY 70 were 31,566. The FY 71 data reflect a 26% increase in the number of tests performed over the preceding year.

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OFFICE OF THE DIRECTOR

SUMMARY

Program direction and administration for the Division of Biologics Standards is based in the Office of the Director, with centralization of administrative procedures relating to personnel, supply and accounting. Major objectives are determined and program responsibilities are assigned to the operating units within the Division. For each major area, staffing patterns are developed and are continually reevaluated in relation to program changes. The required support is determined for each area in relation to the Division's total budget. A major activity of the Director's office is the maintenance of the proper balance between control activities and research programs.

The Information Office is the means for communicating to the public significant aspects of the Division's research and control activities, responding to requests for information from the general public and for reports to other groups within the Public Health Service and the Department, and to Congress.

The tasks of the Biometrics Section represent the performance of work, using already established statistical techniques, for solving problems related to testing of biological products originated in various laboratories. In addition, the Section is involved in studies directed toward perfecting statistical methods used in bioassays and to serving the research needs of the Division. In this respect, it provides biostatistical service for the entire Division.

The Investigational New Drugs Office receives and examines "Notices of Claimed Investigational Exemption for a New Drug," which are required by Section 130.3 of the Food and Drug Regulations, has continued to function within the Office of the Director. During the period covered by this report, 80 "Notices" were received. This brings to 514 the total number of "Notices" received since the Food and Drug Regulations requiring such submissions became effective. The Office has carried on an extensive correspondence to correct deficiencies in submission, to point out possible hazards and to improve investigation protocols. The "Notices" add to the Division's alertness regarding the development of new products. It provides the staff with the opportunity to "tool up" in advance for the formulation of regulations and standards for such new products through this mechanism.

The Office of Scientific Communications performs bibliographic and reference services for the Division, and provides various types of scientific information for members of the staff. Data and information pertaining to licensed biological products and to new products in the offing are of immediate and direct importance to the regulatory and control programs. Since the entire field of biomedical research requires new information retrieval techniques, this means new systems must be worked out for the whole body of knowledge. This requires close contact with information specialists within NIH and in other agencies.

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PERSONNEL ORGANIZATIONAL CHANGES

Several personnel and organizational changes affecting the operation of the Division took place during this reporting period.

Dr. Margaret Pittman retired on January 29, 1971 after 13 years as Chief of the Laboratory of Bacterial Products and 35 years in government service. Dr. Pittman was the first woman to be appointed chief of a laboratory at the National Institutes of Health. She will remain with the Division as a Guest Worker at her pleasure.

Dr. Harold Baer succeeded Dr. Pittman as Chief of the Laboratory of Bacterial Products.

Dr. John N. Ashworth returned to the DBS on March 22, 1971, after five years with Hyland Laboratories.

Dr. Nicola M. Tauraso was appointed Chief of the Laboratory of Virology and Rickettsiology on October [illegible], 1970.

Dr. Vito M. Esposito resigned as Acting Chief of the Diagnostic Reagents Section in the Laboratory of Blood and Blood Products in April of this year.

Dr. Ronald Clark resigned as Chief of the Histopathology Section in the Laboratory of Pathology January 8, 1971.

Seven commissioned PHS officers joined the DBS staff July 1, 1970. Seven more are expected July 1, 1971 to replace a number who are leaving after having completed their obligated service.

The Laboratory of Biophysics and Biochemistry, as such, was abolished on February 3, 1971. This followed the resignation of Dr. Kendall Smith and a number of his assistants who joined the staff of the University of Texas at San Antonio. The remaining staff and resources of the Laboratory were distributed among the other laboratories of the Division with little interruption to the basic programs.

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LICENSING

Licensing actions are based on the recommendations of license review groups of staff members appointed by the Director from the scientific staff concerned with the particular kind of product or establishment.

As of March 31, 1971, 236 establishment licenses were in effect, together with 113 subsidiary establishments and 258 blood bank donor centers. This represents an increase of 1 establishment license since April 1, 1970, the date taken for the last report. This difference is due to the issuance of 8 new licenses and the revocation without prejudice of 7 licenses.

During the 12 months covered in this report, 49 product licenses were issued, 139 were revoked without prejudice at the request of the manufacturers and none were revoked for cause. As of April 1, 1971, 1,246 product licenses were in effect for 262 licensed products.

During this period, all existing licenses for the following products were revoked at the request of the manufacturers:

Chicken Pox Immune Serum (Human)
Diphtheria and Tetanus Toxoids and Poliomyelitis Vaccine
Equine Encephalomyelitis Vaccine (Eastern)
Equine Encephalomyelitis Vaccine (Western)
Measles Immune Serum (Human)
Mumps Immune Serum (Human)
Oxophenarsine Hydrochloride
Pertussis Immune Serum (Human)
Reagent Blood Group Specific Substances A and B
Scarlet Fever Immune Serum (Human)
Scarlet Fever Streptococcus Toxin for Dick Test.

Also, 56 establishment amendments and 163 product amendments were approved during this 12 month period. At the time of this report, there were 31 applications and 24 amendments for establishment licenses and 138 applications and 122 amendments for product licenses pending.

Among the product licenses issued during the year are five which are new products:

Anthrax Vaccine, Adsorbed
Anti-Xg^a Serum
Cryoprecipitated Antihemophilic Factor (Human)
Hepatitis Associated Antibody (Anti-Australia Antigen)
Rubella and Mumps Virus Vaccine, Live.

Also, one manufacturer was authorized to manufacture Red Blood Cells (Human), Frozen as an amendment to their license for Red Blood Cells (Human).

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INSPECTIONS

Forty two members of the scientific staff, selected by the Laboratory Chiefs and the Office of the Director for their special knowledge, are authorized by the Secretary, Department of Health, Education and Welfare to serve as inspectors. These individuals perform inspections as planned and coordinated by the Licensing and Investigations Section of the Office of the Director. Ten additional professional employees assist in the inspections as trainees.

From April 1, 1970, through March 31, 1971, 72 scheduled inspection trips were completed. During these trips 270 licensed establishments, 128 locations, and 138 blood donor centers were inspected for a total of 376 inspections. These inspections required 709 man-days. In addition 30 unscheduled trips were taken to inspect 30 establishments for licensing or reinspection. The unscheduled inspections required 55 man-days.

SUMMARY OF INSPECTIONS

	Scheduled	Unscheduled	Total
Trips completed	72	30	102
Establishments inspected	270	30	214
Locations inspected	128		91
Donor Centers inspected	138		104
Man Days	709	55	588

Also visited were 36 unlicensed establishments that supply material to licensed manufacturers under the short supply provisions of Section 73.240 of the Regulations.

INVESTIGATIONS

Investigations are conducted by inspectors from the Office of the Director. In contrast to routine inspections of manufacturing establishments, which call for expenditure of predictable periods of time, investigations must necessarily be carried out for as long and indefinite a period as is required to obtain all the needed information on a reported or discovered possible infraction of the law. Investigations must therefore be carried out by people who have this duty as their main function. Investigations usually require extensive follow-up as the need is indicated in the course of assembling complete and accurate evidence. If prosecution is necessary, the Justice Department is provided with specialized subject matter guidance, expert witnesses are located and assistance is given in the organization and

preparation of evidence for the most effective presentation. In some situations it is necessary to call upon Laboratory resources for assistance, depending either on the product involved or the nature of the alleged violation.

The Investigations Unit's functions frequently are of mutual interest to other Government agencies. For this reason the Unit maintains a continuing liaison with such agencies as the Justice Department, Department of Agriculture, Bureau of Customs, Post Office Department, Federal Trade Commission, and the Food and Drug Administration.

The Investigations Unit is also responsible for acting on reports of hepatitis as they relate to source material used in the manufacture of biological products or to the final product itself. This requires numerous interviews and extensive record reviews to determine the safety of the products involved.

From April 1, 1970, to March 31, 1971, investigations involved a total of 26.75 man-days off the N.I.H. reservation. This included visiting both licensed and unlicensed establishments for enforcement purposes in addition to interviews and consultations with other federal and state agencies, distributors, witnesses, and others.

Voluntary Compliances

One signed memorandum of voluntary compliance was obtained during this period from a manufacturer of allergenic products who was illegally shipping these products in interstate commerce.

An establishment which had been shipping Anti-Human Chorionic Gonadotropic Serum in interstate commerce without a license agreed to discontinue all shipments.

Customs Detentions

There were four detentions at ports of entry of biological products imported from Europe.

Hepatitis Reports

There were two reports of hepatitis contracted by donors whose plasma was obtained by licensed establishments under the "Short Supply" provisions of the Regulations.

Legal Action Pending

A Criminal Information has been filed by the Justice Department against an establishment which has manufactured and distributed Hepatitis Associated Antibody (Anti-Australia Antigen) in interstate commerce without a license. Arraignment is scheduled for June 28, 1971.

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CONTRACTS PROGRAM

The contracts program is now in its fifth year of expanded operation. To date, approximately thirty-eight contracts have been completed and twenty-three are in progress.

Data obtained from these contracts have proven valuable in providing the Division with information concerning the safety, purity and potency of biological products and leading to the development of standards. Developmental studies designed to produce subhuman primate diploid cell strains have been successful and candidate strains of cells are presently being distributed to interested investigators. It appears possible that such cell strains may serve as substrates for viral vaccines in the future.

The contracts are monitored by Project Officers from the individual laboratories and by members of the Contract Staff of the Office of the Director. The scientific staff of the Division contributes directly to the development and function of the contracts program. This contribution has been of particular value in the initiation, supervision and evaluation of the contract efforts. Often this has involved considerable expenditure of time, made at the sacrifice to the individual's personal research interests.

Details of each contract will be found in "Contract Narratives."

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RESEARCH ACTIVITIES

This summary of the programs of the laboratories reflecting the interests of Division scientists will be identified with the laboratory of the principal investigator. There is, however, much inter-laboratory collaboration.

SECTION ON EXPERIMENTAL VIROLOGY (OD)

This Section is concerned with certain aspects of viral carcinogenesis, particularly with the circumstances under which neoplastic changes in cells can be enhanced or suppressed.

Simian virus 40 (SV40) was repeatedly recovered from SV40 induced tumors which had been transplanted over 200 times in adult hamsters. The virus was shown to be synthesized by the hamster cells and could not be recovered by conventional methods. Most transformed or neoplastic cells were free of infectious virus.

Since some of the hamster tumor cells are virus producers, it can be speculated that some human cancer cells may also produce infectious virus.

These studies have shown that repeated injections, of a non-oncogenic variant of adenovirus type 12, protects hamsters against tumor development if the newborn are infected with the oncogenic source strain.

Preliminary experiments suggest that the non-oncogenic strains of adenovirus type 12 are unable to stimulate transplant immunity which suppresses the growth of transplanted tumors. Attempts to develop a non-oncogenic strain of SV40 have not been successful.

Attempts to induce or enhance oncogenicity in animals with non-oncogenic adenovirus strains or with herpes-like virus have also been unsuccessful.

This program provides information on immunity in relation to tumor development and contributes to the understanding of possible hazards in biological products under development.

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LABORATORY OF BACTERIAL PRODUCTS (LBP)

Dr. Margaret Pittman retired from LBP, and Dr. Harold Baer has been appointed to succeed her. No major changes are anticipated in either the research or collaborative programs of the Laboratory.

The research program of LBP involves a variety of projects designed to investigate basic mechanisms of allergy and infectious diseases, as well as applied projects designed to develop new or improved standards or testing procedures for products subject to license under the Public Health Service Act.

In addition, the staff reviews license applications, amendments, labels and IND reviews. It also inspects licensed establishments and those establishments seeking a license.

Allergenic Extracts; A potency assay for short ragweed extract for practical use has now been developed, based on the qualitative delineation of the presence of antigen E. Manufacturers of allergenic extracts are now engaged in a collaborative project with DBS, and it appears that the first specific potency assay for an allergenic extract will be available within the year. This is an excellent example of a basic research project leading to an applied test applicable to DBS activities. Work on grass pollen extracts is still in progress in collaboration with a group at Johns Hopkins University, and it appears that a similar test may be devised for these extracts based on the skin-reactive group I allergen.

More sensitive and specific techniques for the assay of allergens are needed, and studies will have to be carried out for this purpose.

Studies on the relationship of mite antigens to the allergenic activity of house dust extracts are yielding interesting results. Many house dust extracts contain mite antigens, but, curiously, the antigens seem to be either of different kinds or in different molecular forms since different immunologic tests are required for their detection.

Poison Ivy and Poison Oak: Because of the use of poison oak and poison ivy extracts administered by varying routes to effect desensitization, much effort was expended on studying the physiological and immunological effect of administering pentadecylcatechol, one of the essential elements of poison ivy extracts, and other sensitizers by varying routes. These materials are rapidly absorbed from the skin and appear in the blood stream, excreta and most organs. Whether or not sensitivity or tolerance results is a complex matter, and exact conditions leading to one or the other cannot yet be accurately defined. This study is continuing.

Cholera and Cholera Antigens: Since current cholera vaccines do not provide long-lasting immunity, a search has been made for other than a whole organism vaccine. Cholera toxoid, prepared from cholera toxin, in preliminary studies appears to hold promise as a new immunizing agent. It is relatively easy to purify the toxin. The first studies in humans indicated that the toxoid gave many local reactions. Experiments in guinea pigs and monkeys show that local reactions can also be reproduced and may possibly result from the presence of cholera toxin in the toxoid.

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Typhoid Vaccine: The role of the various antigens of *Salmonella typhosa* in inducing immunity in man is still an unresolved matter; the role of Vi antigen is still not clear. A typhoid vaccine after the enzymatic removal of the Vi antigen was shown to confer specific immunity in mice to *S. typhosa* which was transferable with serum. Therefore, there are mouse protective factors in this vaccine which are not Vi antigen. As an aid in this study, a new method of isolating and measuring Vi antigen was developed; the product is free of somatic antigens.

Pseudomonas Vaccine: Pseudomonas vaccine, a mixture of soluble antigens from seven different serologic types, is a product proposed for use in certain circumstances, that is, persons suffering severe burns. It has been demonstrated that the product is largely, if not exclusively, endotoxin. Nevertheless, it can confer protection to mice challenged with living organisms, and this can form the basis for a potency assay if this vaccine should become a licensed product.

Mycoplasma: It has been shown that many tissue culture cell lines are contaminated with mycoplasmas. Studies with such cell lines have now shown that interferon production may be reduced while virus yields are increased. A new simian species of mycoplasma has been isolated, as well as some new species from bovine plasma, used in tissue culture media, which is frequently contaminated with these organisms.

Tetanus Studies: Tetanus toxin appears to be a mixture containing at least a neurotoxin and a hemolytic toxin, the latter usually referred to as tetanolysin. The tetanolysin can destroy not only red cells but also platelets. The physiologic effects resulting from cell lysis are under investigation.

Pertussis Immunoglobulins: A study was carried out using the mouse lung infection model to evaluate the role of various immunoglobulins in protection against infection by *B. pertussis*. Tracheobronchial washing and serum revealed a significant rise in IgA capable of binding to *B. pertussis* organisms, which correlated with clearance of the infection. This suggests that IgA may be an essential factor in pertussis immunity.

Tuberculins: There are many species of mycobacteria that can induce sensitivity in humans. The taxonomy of these organisms is not well established nor is it yet possible to clearly distinguish the causative agent for specific sensitivity induced in human beings by natural infection. Immunoelectrophoretic studies of culture filtrates showed that these organisms share many antigens. However, skin tests using culture filtrates as antigens in homologously and heterologously sensitized guinea pigs seem to provide a degree of specificity adequate to differentiate the sensitizing antigens. The skin reaction eventually may also prove to be an important aid in improving the taxonomic status, especially of the avium-intracellulare group.

Studies with tuberculoprotein and carbohydrate from a BCG culture filtrate showed that the protein could induce lymphocyte transformation and inhibit the migration of peritoneal macrophages. The polysaccharide, however, could only induce inhibition of macrophage migration. Not only does this provide a striking difference between these two *in vitro* tests, but may aid in elucidating their mechanisms.

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LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)

The research program of the Laboratory included areas directly related to the control responsibilities of each of the individual sections--Blood and Blood Derivatives, Coagulation, and Diagnostic Reagents.

Ultracentrifugal analyses of a sampling of in-date lots of Immune Globulin (Human) products revealed a considerable number which contained degraded (3.5 S) material. A prospective study involving various storage temperatures and the use of urokinase as a plasminogen activator has therefore been initiated in an attempt to devise a test by which the stability of such products can be predicted. Concurrently with this study, various methods have been examined to determine their suitability for detection and/or quantitation of the proteolysis. These have included 1) immunoelectrophoresis, 2) ultracentrifugation, 3) disc electrophoresis, 4) acrylamide gel electrophoresis in sodium dodecylsulfate, 5) exclusion chromatography, and 6) isoelectric focusing. In a direct comparison the relative sensitivities were found to be 1)> 4) >2)>> 6). The ability of mild heating to increase the stability of Immune Serum Globulin (Human), previously demonstrated in an "accelerated storage" study at 24° C, was confirmed for storage at 5° C. These results prompted attempts to determine whether a similar effect is exerted on immunoglobulin G in whole serum and to learn what effects heating has on the characteristics of Immune Serum Globulin (Human). The former investigation has been partially completed; the latter revealed that mild heating brought about a decrease in the amount of IgG dimer. This decrease, which could occur in the absence of fragmentation and aggregation, was shown to reflect a reversible, temperature-dependent monomer--dimer transition.

Examination of certain blood products, viz., Normal Serum Albumin (Human), Immune Serum Globulin (Human), cryoprecipitates, and Antihemophilic Factor (Human), by immunological techniques has led to the following observations. Products of placental origin are contaminated with at least one placental component. In addition, Immune Serum Globulin (Human) prepared from placentae contains appreciable quantities of blood group substances (mostly group B substance), whereas immune globulins from venous or mixed sources contain much less. Immune Serum Globulin (Human) and cryoprecipitates contain low levels of isoagglutinins; in some cases Antihemophilic Factor (Human) may contain much more. The latter finding has led to the establishment, by the manufacturer, of upper limits for the level of isoagglutinins in Antihemophilic Factor (Human).

Work on the coagulation system has resulted in the preparation of human Factor X which contains little Factor IX and is virtually free of Factor VII, has disclosed a congenitally abnormal human fibrinogen which exhibits delayed release of fibrinopeptides A and B (the first such dysfibrinogenemia reported), and has demonstrated that a major biochemical defect in human Factor XIII deficiency is the inability to form ε-(γ-glutamyl) lysine crosslinks.

Antilymphocytic sera have been tested in a variety of systems. It has been found that, depending on the species of origin, these sera may be species-specific or both species- and strain-specific. Similarly, test systems, including a cytotoxicity test developed in this Laboratory, for typing human histocompatibility antigens, are being evaluated.

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LABORATORY OF CONTROL ACTIVITIES (LCA)

I. Mission

The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity and potency. The Laboratory is composed of three sections: Analytical Chemistry, Control Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the approximate number tested by the Laboratory is 300 for sterility, 200 for safety, 100 for potency, 100 for chemical, 50 for pyrogen and 40 for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Research is conducted on development and modification of control tests on biological products.

(b) To develop, maintain and distribute biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with published standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) In additon to the above, the Laboratory shares responsibility with the other Laboratories in the Division in developing standards and regulations, performing inspections, reviewing license applications and maintaining a technical liaison with the World Health Organization, other national control agencies and recognized authorities in the field of biological products.

II. Product Testing

Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serve as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Control Test Section of the Laboratory of Control Activities provides regular cooperative pyrogen testing services to the Clinical Center Pharmacy, Blood Bank and Media Department and sterility testing for the Clinical Center Pharmacy and Blood Bank.

A summary of 12,509 tests performed by the laboratory during a 12 month period (April 1, 1970 through March 31, 1971) in making the determination that products meet prescribed standards is shown in Table 1.

III. Standard Preparations

An integral part of the system of manufacture and control of biological products is the application and use of standard preparations. By the use of such standards, potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the laboratory to provide these standard and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of standards and references it was necessary to prepare and calibrate during the past year the following lots of materials: 14 antitoxins, 3 serums, 3 vaccines, 110 miscellaneous preparations. In addition to these materials prepared by the laboratory the following lots of materials were purchased from commercial sources and finished by the laboratory: 3 virus vaccines, 6 bacterial vaccines, 4 skin test antigens, 2 blood typing sera, and 1 blood group substance. A total of 537 tests were required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted *in vivo* and *in vitro* tests.

The technique for freeze-drying is a key procedure applied in the preparation of standard and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year.

Cultures	2,219	ampules
Serums	2,016	"
Vaccines	1,133	"
Viruses	1,450	"
Tuberculin	3,196	"
Plasma	250	
Blood Group Substance	501	"
Toxin	29	"
Total	10,794	"

The following standard and reference preparations, consisting of a total of 1,022 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U. S. licenses and qualified foreign laboratories.

Australia Antigen	624
Antitoxins	456
Serums	12,387
Vaccines	2,218
Toxins	626
Bacterial & Viral Cultures	323
Allergens	457
Viruses	1,042
Opacity Standard	61
Globulins	125
Blood Products	1,099
Miscellaneous	53
Total	19,471

Approximately 120 different standards, references, and related control preparations are maintained for distribution.

IV. Research and Development

While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by laboratory personnel during this fiscal year.

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(a) Opacity Standard: The U. S. Opacity Standard used in the standardization of cell concentrations of bacterial vaccines and of bacterial challenge suspensions used in potency tests is composed of finely divided glass particles suspended in water. During use and storage the opacity of the standard gradually lessens due to changes in particle size and adherence of particles to the walls of the container. Another undesirable feature is the difficulty of reproducing exactly the properties from lot to lot. For example the present international preparation differs from all previous international preparations as well as from the U. S. Standard. This is causing serious problems in laboratories throughout the world.

Tubes have been prepared with resin containing a homogenous suspension of particles of titanium dioxide. It was discovered that a concentration of the particles which yields light transmission equal to the U. S. Opacity Standard when compared with a nephelometer does not appear the same when compared by the unaided eye. Two sets of tubes have been prepared, one being equal to the Standard by instrument and the other by eye. Stability studies are in progress to determine the practicality of this type of solid containing static particles for estimating concentrations of bacterial cells in a liquid.

(b) Pseudomonas Vaccine: Studies in collaboration with the Laboratory of Bacterial Products have continued to provide basic data relative to the development of a Pseudomonas Vaccine. Studies have been made on the characteristics of several strains of the organism in relation to vaccine production, testing and use.

(c) Smallpox Vaccine: A broad program related to controlling the purity and potency of smallpox vaccine is continuing. A collaborative study with a large manufacturer of smallpox vaccine relative to the control of potency of vaccine with varied volumes of inoculum on the chorioallantoic membrane (CAM) of chicken eggs was carried out. The results of the study showed that licensed manufacturers of smallpox vaccine could use either 0.1 ml. or 0.2 ml. inoculum when testing vaccine potency by the CAM procedure.

(d) Sterility Testing: Several aspects of sterility testing are being studied with the object of improving the validity of sterility tests and reducing the time, volume of medicum and cost involved in performing each test. New and modified media are being studied using selected microorganisms having peculiar growth characteristics. The use of membrane filtration for testing live virus vaccines containing antibiotics is being studied. An improved cutter for dividing the membrane in two exactly equal portions was developed.

(e) Tuberculin: Three collaborative studies with CDC have been conducted to study the stabilizing properties of Tween 80 in dilute solutions of tuberculin and to obtain a better understanding of the relationships between tests in sensitized guinea pigs and results of clinical use in man. As a result of these studies CDC is now distributing Tuberculin, PPD at the 5 T.U. strength which contains 5 ppm of Tween 80. Clinical studies are in progress with portions of large master batches of PPD produced by three manufacturers. The objective of these studies is to characterize these batches in order to evaluate better the maning of reactions obtained in different populations. A large lot of the atypical mycobacterial strain, Battey, was prepared and freeze-dried for use as the U. S. Standard and for distribution from CDC for clinical testing. Studies on all aspects of tuberculins are being pursued actively.

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(f) Typhus Vaccine: Vaccine prepared in a series of four two-fold levels of activity have been obtained under contract with a licensed manufacturer. The four preparations have been tested in laboratory studies for safety, potency and purity. The data are being assembled to be submitted as an application for clinical studies as an investigational new drug. It is planned to study the response in man to different levels of vaccine potency in order to establish the optinum potency for Typhus Vaccine. Modifications to the animal potency test are being developed in order to improve the sensitivity of that test. The project on Typhus Vaccine is being conducted in collaboration with the Laboratory of Virology and Rickettsiology.

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LABORATORY OF PATHOLOGY (LP)

The Laboratory of Pathology has a continuing program to study the pathogenesis of infectious diseases and immunologic processes using animal models. This program is designed to meet the overall needs of the Division in regard to the safety and efficacy of new biological products and also those which have been licensed for many years. The scope of the research activities of the laboratory has broadened considerably over the past several years. This is particularly so since the scientific personnel of the laboratory have interests which although broadly related to pathogenesis of infectious diseases, are related to biochemistry, virology, immunology, and histology as well as pathology.

The personnel of the laboratory has undergone considerable change within the past year. Dr. Ronald Clark, who had been in the laboratory for eight years and had essentially received most of his scientific training in that time (having received his doctorate while working here) left in December to join an academic faculty. Similarly, Dr. Deborah Carpenter, the only other fully trained pathologist decided to join the faculty of the Georgetown University School of Medicine. On the bright side, however, Dr. Stanley Singer who had left in 1969 to resume residency training, returned to the Division in August, 1970 and immediately resumed extremely productive studies. Dr. John Petricciani who has responsibilities for many contracts has become a member of the permanent staff. There is, however, a need for another individual with experience in pathology since requestsfor collaborative pathology studies are increasing rapidly.

Research Activities

The research aspects of neurovirulence testing of viral vaccines have become secondary to the testing procedures. However, past experience has shown that such tests must never be considered routine or the meticulous care needed to assure the safety of viral vaccines will be lost. This is particularly important at present, since consideration is being given to approval of production of Poliovirus Vaccine, Live, Oral using human cell strains as a substrate (Project Number 41, p.108).

Recent discussions concerning the routine use of Smallpox Vaccine in the United States have led to the studies of the pathogenesis of various strains of vaccinia virus in monkeys. (Project Number 112, p.115)

Dr. Lorenz and other members of the staff of the Laboratory of Pathology and of Laboratory of Viral Immunology have in progress numerous studies of candidate agents of human hepatitis. The marmoset, *Saguinus mystax* appears to be a useful animal model for studies of the pathogenesis of infectious (short incubation) hepatitis. To date, only the chimpanzee and man are

susceptible to serum (long incubation) hepatitis. Studies of serum hepatitis in chimpanzees are being performed under Contract No. NIH 71-2040 with Hazleton Laboratories. These studies complement the work on infectious hepatitis in marmosets. (Project Number 133, p. 117).

Dr. Singer has resumed his investigations of the interaction of viruses, cells and mycoplasma and, in addition, with Dr. Hardegree of LBP, has studied the induction of interferon by some licensed biological products, namely some allergenic extracts and bacterial vaccines with no U. S. standard of potency. (Project Number 201 and 200, p. 123).

Dr. Petricciani in collaboration with investigators in NIMH has been studying various cell strains as to absence or presence of several enzyme activities and has shown that, by use of specific microorganisms, certain enzyme defects can be corrected. (Project No. 162, p. 122).

Dr. Shabo has continued and expanded studies begun by Dr. Ronald Clark who recently left NIH. These studies relate normal anatomic pathways of the central nervous system to barriers to viral infection and to immunologic mechanisms and suggest methods for allowing drugs and antiviral substances to enter the cerebrospinal fluid and perhaps the central nervous system tissue itself. (Project No. 202, p. 127).

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program as all other research activities is under the direction, supervision, and coordination of the Chief of the Laboratory.

Further, the Chief of the Laboratory has directed most of her own research efforts in the general field of the pathogenesis of infectious diseases with particular emphasis on oncogenic viruses. The pathogenesis of the particular tumors induced, the effect of altered immunologic mechanisms, and the interaction of these viruses on each other are all being studied. At the present time the possible oncogenicity of herpes viruses is of particular interest (Project No. 44, p. 110).

Collaborative (Contracts) Activities

The Laboratory of Pathology plays a very active role in the collaborative program of the Division, since most if not all contracts have aspects relating to pathogenesis and require pathologic interpretation. The Chief of the Laboratory of Pathology works very closely with the various project officers, the Contracts Operations Officer, and the Director, DBS in helping to plan and execute various projects and to review progress reports of those contracts already in existence. She also reviews histologic material from contract work as necessary. Furthermore, she serves as a liaison between Collaborative Program Directors of other Institutes, the Assistant Director of NIH for Collaborative Research, and the Division.

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Dr. Petricciani has served as Project Officer for four contracts concerning the development of diploid cell strains from sub-human primates and another contract concerning survival of donor white blood cells in recipients. He also served as an Assistant Project Officer for one contract while Dr. Lorenz served as Assistant Project Officer for three contracts. Dr. Chisari serves as Assistant Project Officer for one contract.

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LABORATORY OF VIROLOGY AND RICKETTSIOLOGY (LVR)

A. ADMINISTRATIVE ASPECTS

Introduction.

During this fiscal year, continued emphasis was placed on the development of a program which would coordinate straightforward control with control-related research responsibilities. Progress has been made in phasing out old programs efficiently and developing new meaningful ones. However, the over-all operations of the Laboratory have been adversely affected by two events: Firstly, the dragging out of a poorly conducted EEO investigation caused considerable disruption of the operations of the Laboratory. Although the initial complaint was filed on 10 March 1970, as of the date of this report, the problem remains unresolved. Secondly, an amateurishly conducted formal review of the grievances of one employee was similarly dragged out resulting in additional disruption of the operations of the Laboratory.

Mission Statement. The Laboratory encompasses a broad program of research concerning the prevention and treatment of viral and rickettsial infections in man. Specific emphasis is placed upon problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. Constant liaison with the Division's other Laboratories achieves a balance in the harmonious execution of the DBS control and research responsibilities.

Personnel Changes. The full-time employee situation remained quite stable. Only two individuals (GS-5 and GS-3) were hired into positions made vacant because of death and resignation respectively.

Organizational Changes. The Laboratory of Virology and Rickettsiology (LVR) is now composed of an Office of the Chief, the Virology and Rickettsiology Section, and the Cell Biology Section. The organizational components of LVR have remained unchanged.

The Virology and Rickettsiology Section is responsible for conducting basic and applied research concerning the prevention and treatment of viral and rickettsial infections in man, with specific emphasis on problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. This Section is composed of the following 7 units: Immunoserology, Avian Leukosis, Immunofluorescence, Respirovirus, Arbovirus, Rickettsial, and Viral Pathogenesis.

The Cell Biology Section is responsible for maintaining a facility to provide cell cultures to DBS investigators and for conducting an ongoing research program concerned with problems which might arise from the use of cell cultures for preparing virus and rickettsial vaccines. This Section is composed of the Cell Production and Cell Research Units.

B. RESEARCH ACTIVITIES

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constitutents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

This Laboratory was unable to confirm previously published data which showed that the A2/Aichi/2/68/X-31 so-called recombinant influenza virus strain had a growth potential far superior to the A2/Aichi/2/68 alleged parental strain. In contrast, it was demonstrated that, under optimal conditions, the so-called recombinant X-31 strains were able to grow only 1.5 times greater than the A2/Aichi/2/68 alleged parental strain. These results have been confirmed by all of the U. S. licensed influenza vaccine manufacturers and another independent investigator.

Research towards evaluating diluents and automatic diluting and pipetting machines in influenza serology led towards the development of a reliable and reproducible semiautomatic method of performing influenza serology.

A quantitative radial immunodiffusion test to detect soluble residual host protein in chicken embryo-derived vaccines has been developed. This test will be employed on all influenza vaccine lots submitted for release in the hope that progress in eliminating residual host protein in inactivated influenza vaccines will be possible.

Volunteer studies were performed to make comparative studies on monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. The incidence of reactions was lowest among volunteers receiving the more highly purified vaccines, such as zonal purified, ether-extracted hemagglutinin and filter-purified Sharples vaccines. Interestingly, however, there was no apparent relationship between reactogenicity and measureable avian antigen and protein nitrogen.

Surprising as it may seem, even after influenza vaccine has been in continuous use for almost 25 years, its degree of efficacy is in doubt. This doubt has arisen from the confusion of a multitude of contradictory reports in the literature. A critical review of this literature has revealed that most investigators have not appreciated the fact that until most recently we

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lacked reliable methods for quantitating the antigen content of vaccines. Clinical evaluation studies must be supported by a critical laboratory analysis of antigen content, and a lack of knowledge of the latter precludes any meaningful conclusion of efficacy studies in man.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

Studies of the antigenicity and safety of the avian leukosis virus--(ALV)-free yellow fever (YF) vaccine primary seed have been completed. All of the original objectives of the YF project were realized. Candidate ALV-free primary and secondary YF vaccine seeds were developed by ridding the 17D virus vaccine seed of its ALV contaminant. The passage level of the candidate ALV-free primary seed (lot no. 17D-51) is the same as the present primary vaccine seed (lot no. AB-237) which has been employed for vaccine production for the past 25 years. The safety and antigenicity of the ALV-free YF virus strain in monkeys and man have been established. A recommendation has been made to adopt the use of the new ALV-free YF primary vaccine seed.

Progress has been made towards the development of a cell culture derived YF vaccine. Adequate virus titers were achieved in virus-infected primary chicken embryo cell cultures. Neurovirulence tests comparing the cell culture derived virus and the conventional chicken embryo derived vaccine showed these to be indistinguishable. Plans include antigenicity studies in monkeys and a clinical evaluation in man.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Recently, the fluorescent antibody (FAB) test has been used to detect the virus causing Marek's disease, a contagious neuroproliferative disorder which is prevalent in chicken flocks in this country. From what is already known about Marek's virus, it is highly unlikely that this virus could survive the commonly employed procedures for preparing live virus vaccines. We will soon be able to examine finished vaccine and remove the speculation concerning the potential contamination of egg derived vaccines by Marek's virus.

The Avian Leukosis Unit of this Laboratory has been responsible for monitoring the NIH ALV-free chicken flock to insure that the flock remain free from these viruses. From collaborative studies with a USDA geneticist it was recently discovered that the NIH flock was not homogeneous genetically and that, by and large, the flock was resistant to avian leukosis subgroup B viruses (i.e., C/B). We have been able to identify and segregate some chickens with respect to their genetic susceptibility. A system of cross-breeding has been established and the embryos of trap-nested eggs are currently

being screened to determine their genetic status. It is our hope that, eventually, we will be able to establish a flock which will be susceptible to viruses of all subgroups (i.e., a C/O flock). This will enable us to perform more easily our control responsibility to insure that vaccines produced in chicken embryo derived tissues are free from contaminating avian leukosis viruses. The maintenance of an avian leukosis virus genetically susceptible flock is a time-consuming, lack-lustre chore which is not fully realized by egg users, but which is nevertheless singularly necessary for certification of a "gold-bar" flock.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed towards developing a candidate lot of inactivated Typhus Vaccine for use as a reference antigen. Lots of commercially produced Typhus vaccine believed suitable for a reference vaccine have been obtained and tested by the Laboratory of Control Activities (LCA), DBS and by the manufacturer. The four candidate lots of vaccine have passed the safety, purity and potency tests ordinarily required for licensing of Typhus Vaccine. Clinical trials have been initiated to evaluate the reactogenicity and antigenicity of these preparations in volunteers.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of LVR became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains of most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB, DRS (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July 1964).

We have since shown that four subsequent epizootics (namely, at the National Center for Primate Biology, Davis, California in September 1967, and at Sussex, Great Britain in December 1966, January 1968 and again in February 1969) were caused by the same virus. The significance of knowing more about potential hazardous diseases in monkeys is obvious. In addition, simian hemorrhagic fever provides a natural and safe model for the study of the pathophysiology of hemorrhagic fever. It is hoped that our studies might lead to a better understanding of hemorrhagic fever in man, and eventually might result in development of successful therapy for a disease with such high mortality rates.

Immunobiological Studies. Of major concern in studies of delayed hypersensitivity has been development of an *in vitro* test, particularly for man, and the discovery of the factor or factors that confer a passive hypersensitivity state. Previous studies using human RNA species *in vitro*

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have shown the transfer of an immunological message to previously nonsensitive human lymphoid cells. Defining the mechanism of activity is paramount for evaluating RNA as a biologic product.

Studies have included: (1) isolation, purification and immunological characterization of cell antigens; and (2) an attempt to define the immunologically active molecule in a situation where RNA extracts are able to transfer antigen recognition to host lymphoid cells with which the RNA extracts incubate. These investigations show promise in such areas as: (1) identification of culture variability and antigenicity; (2) control and standardization of transplantation immunology; (3) selectivity and potency evaluation of antilymphocyte globulin; (4) tumor cell identification and standardization; and (5) a better understanding of autoimmune disorders.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis) and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Inconsistencies or failures in isolating causative agent in acute and chronic diseases of the central nervous system (CNS) constitute a major handicap in tracing the origin of the disease and relating it to certain pathogenetic events. Studies were performed to determine optimal procedures for isolation of viral agents from the CNS. Application of new isolation techniques led to rapid isolation of measles virus from monkey brains in acute model infections.

Additional studies were performed evaluating circumstances under which measles virus displays neurotropic properties and is capable of establishing an acute or chronic progressive disease of the CNS. Individual measles viruses displayed marked differences in their cultural and physico-chemical properties. Neurotropism of a strain was best related to reduced heat stability, resistance to freeze-thaw damage and susceptibility to inhibitors from brain tissue.

Knowledge of the biological properties of the neurotropic strains as well as the character of the host reaction are important for a better understanding of potential preventive or curative measures.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) developing new and improved techniques and methods for large-scale production of cell cultures; (2) developing additional cell culture systems as potential substrates for vaccine purposes; (3) studying selected biochemical parameters of cells in culture as may be related to viral susceptibility; and (4) studying factors which may influence somatic variation and modulation in cell culture systems.

As a result of some of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

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LABORATORY OF VIRAL IMMUNOLOGY (LVI)

Laboratory Chief's Summary of Research

The Laboratory of Viral Immunology conducts a research program that tends to be applied in nature and product or disease oriented. Our goal is to maintain a program that will meet current and anticipated needs of the Division in the area of virology. The Laboratory conducts direct research, assists in collaborative research (guiding of contract-supported activities) and is involved in the review of scientific data submitted to the Division in its capacity as a regulatory agency. These reviews deal with existing biological products, license applications for new products and investigations covered by investigational new drug exemptions. The discharge of these responsibilities involves laboratory and clinical evaluation of products as well as test development.

Certain LVI activities are highlighted in the following paragraphs:

Rubella. During the past 1-1/2 years three manufacturers were licensed to produce live rubella virus vaccines. As a result of advisory committee recommendations for prompt and extensive vaccine use there has been a massive effort resulting in the production, distribution and administration of more than 25 million doses over a period measured in months. The LVI and DBS, having anticipated this thrust, have handled the increased load of protocol review and testing of individual lots without difficulty. Likewise the Laboratory has recognized a need to maintain a research program capable of adding to the existing data relating to safety and effectiveness as the base of clinical experience has increased from approximately 100,000 (prelicense experimental use) to many millions.

This research effort has involved both direct investigation and collaborative activities with the Center for Disease Control and other organizations. The level of vaccine-associated rubella-like reactions is more precisely defined. Encouraging information pertaining to vaccine-induced protection and duration of immunity has been analyzed and published as have new data confirming the ability of the attenuated viruses to infect the placenta (as a result of inadvertent immunization during pregnancy).

Combined Vaccines. The availability of an ever increasing array of live virus vaccines has led to appreciation of the potential advantages of developing polyvalent products. This is not a matter of mere convenience but rather a mechanism by which costs can be reduced and a higher percentage of the susceptible population effectively immunized.

Modern work on combined vaccines was in part stimulated by the investigations of our group in 1962 (Meyer, H. M., Jr. et al.: Response of Volta Children to Jet Inoculation of Combined Live Measles, Smallpox and Yellow Fever Vaccines, Bull. WHO 30:783-794, 1964). Two mixtures tested in the clinical trials, measles-smallpox and measles-smallpox-yellow fever, appeared promising for use in countries still dealing with the problems of endemic smallpox and yellow fever. Additional research by an American pharmaceutical group led to license of a combined live measles-smallpox vaccine in 1967.

More recently the development of a live mumps vaccine and subsequently a live rubella vaccine made it possible to explore the feasibility of preparing other combinations of attenuated viruses appropriate for more universal use. The principle theoretical obstacles are that multiple simultaneous infections might either enhance the reactivity of one or more components or decrease the immunizing effectiveness through viral interference (Meyer, H. M., et al.: Combined Measles-Smallpox and Other Vaccines, First International Conference on Vaccines Against Viral and Rickettsial Diseases of Man, Washington, D.C., Nov. 7-11, 1966, PAHO and WHO Scientific Publication No. 147, 336-342, May, 1967). Anticipating the course of pharmaceutical research we have maintained an active laboratory program in this area and recently conducted a series of clinical trials of products proposed for license. The overall data generated by the manufacturer, ourselves and other investigators proved the safety and potency of this second generation of polyvalent products. Thus, measles-rubella, mumps-rubella and measles-mumps-rubella vaccines were licensed during the current year. We anticipate extensive use of these new products in the American pediatric population.

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Hepatitis. The LVI has a major commitment to the study of this virus-induced syndrome. Great strides have been taken over the past year in developing methods of assaying the hepatitis associated antigen (HAA) of long incubation period hepatitis ("serum hepatitis"). Likewise new techniques now make it possible to detect anti-HAA antibodies in the majority of persons infected.

Investigations supported by a DBS contract have evaluated the significance of HAA positively in donor blood in New Jersey. In these studies the sensitivity and specificity of antigen assay procedures have been defined by following the recipients of tested blood for the development of hepatitis. More than 200,000 donor units of blood have been tested with consistent evidence that commercially procured blood was more frequently HAA-positive than voluntary donor blood. The correlation of HAA with subsequent infection of the recipient was striking. Fully 69% of patients given HAA-positive units developed liver derangement or other evidence of infection as compared to only 27% of recipients of HAA-negative blood.

Translating these research findings into patient benefit has the highest priority. The first step consisted of developing standards and subsequently licensing commercial preparations for use in the detection of HAA in human blood. As these new reagents become generally available it will become possible to require the routine screening of all donor blood so as to exclude those units found to contain HAA.

To insure that testing methods had maximum sensitivity and specificity the DBS entered into a collaborative comparison of assay methods with the Committe on Plasma and Plasma Substitutes of the Division of Medical Sciences, NAS-NRC and the Center for Disease Control. These studies along with the development of standard negative and positive reagents should speed implementation and acceptance of the new techniques.

The hepatitis program has obtained other data of wide clinical, epidemiological and virological interest. For these items, such as a possible association between HAA and hepatic carcinoma, the reader is referred to the individual project report (DBS 137).

Cytomegaloviruses. These highly species-specific members of the herpes group of viruses are of considerable interest. The CMV of man are important pathogens producing maternal-fetal disease (with congenital defects) as well as other syndromes. The LVI has increased its commitment to CMV investigation during the period of this report. This work has had two objectives. The first involves the development of a subhuman primate model for study of the pathogenesis and ecology of CMV. This long-term approach will hopefully, in time provide data translatable into methods for controlling CMV disease of man.

The second aspect of the project has been to obtain information relating to the potential for contamination of poliovirus vaccines (produced in monkey kidney cell cultures) with simian CMV. Sero-epidemiological studies coupled with direct assay of monkey kidney cultures confirmed that most simians do in fact harbor small amounts of CMV. Direct tests on vaccine lots for CMV were, however, consistly negative. Subsequently experiments were conducted in which simulated vaccine was produced in cultures deliberately contaminated with CMV. The conclusions from these investigations have been that trace amounts of CMV may be present in monkey cells used for vaccine production but that infectious CMV probably rarely if ever reaches final vaccine due to its slowness of replication, cell-association and instability. Other studies have failed to detect simian CMV antibodies in monkey handlers (exposed to animals known to be excreting virus) and in recipients of polio vaccines.

While no evidence of vaccine contamination was obtained this is regarded as an undesirable situation. It should encourage the consideration of alternative cell sources for polio vaccine production.

Cell Culture Studies. Cultured human and animal cells are one of the more important tools of the medical investigator. They have diverse application in virology, oncology, genetics, etc. as well as practical uses such as the production of vaccines. Many LVI projects could be considered to be "cell culture studies". Four lines of investigation are, however, particularly appropriate for mention here.

The first concerns the extensive work of the LVI on cultures prepared from tissues of domestic and wild rabbits. This project was undertaken in 1969 in anticipation of the license of the first virus vaccine to be produced in rabbit kidney cell cultures (Cendehill strain rubella vaccine). Our investigations continued into the present year and were important in documenting the safety of the vaccine (Details can be found in individual project reports, DBS 49 and DBS 86 for the current and previous years).

A second avenue has concerned the documentation and/or development of diploid strains of human and animal cells potentially suitable for vaccine production. These investigations have been supported primarily by contract and have increased in scope during the past year. They include studies of human and animal karyology and the development and testing of diploid cell strains from monkeys and rabbits. The results to date have been promising and suggest that either human or simian diploid cells or both might be considered as possible substitutes for primary monkey cell cultures in polio vaccine production.

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The third area has dealt with the question of viral etiology of rheumatoid arthritis (RA). Studies were initiated because of the observation that the joint involvement sometimes induced by rubella or rubella vaccination in many ways mimics RA. Joint fluids and synovial biopsies from RA patients were used to establish growing cells in culture. Cultures were subjected to a number of tests searching for evidence of overt or masked rubella viruses and other viruses. No evidence to date has indicated that rubella viruses bear any relationship to RA or other chronic joint disorders. (Details of the work are summarized in individual progress report DBS 183).

Finally cell cultures have been used in a wide-ranging series of genetic investigations conducted in collaboration with teams in NIMH, NHLI, NIAMD, NINDS and several universities. Determining the metabolic profile of cells cultured from patients with heritable diseases is proving useful in defining the pathological mechanisms operative in these conditions. Such studies may in time provide clues enabling prevention or therapy.

Viruses and Cancer. Over a span of years scientific attention has progressively focused on the role of viruses in the etiology of human cancer. Further progress in this area could in time lead to the development of new biological products. For this reason one component of the LVI has for several years had a full time commitment to research in viral oncology. Their work with the Epstein-Barr virus (EBV) has resulted in several exciting discoveries.

This herpes-group virus originally recovered from cultured cells derived from Burkitt's lymphoma is suspected of causing infectious mononucleosis and perhaps other lymphoproliferative diseases of man. As described in the individual progress report (DBS 127), they found that EBV infection of normal buffy coat cells *in vitro* led to stimulation of DNA synthesis. This characteristic has been found in animal tumor viruses but has not been associated with non-oncogenic viruses. Subsequently it was shown that EBV infection of normal lymphocytes led to transformation into continuous lymphoblastoid cells. Finally, EBV-transformed cells when inoculated into immunosuppressed newborn mice caused uncontrolled, metastasizing lymphoblastoid cell proliferation with death of the animals.

Since most humans are infected with EBV at some time during their life, these observations are of considerable interest.

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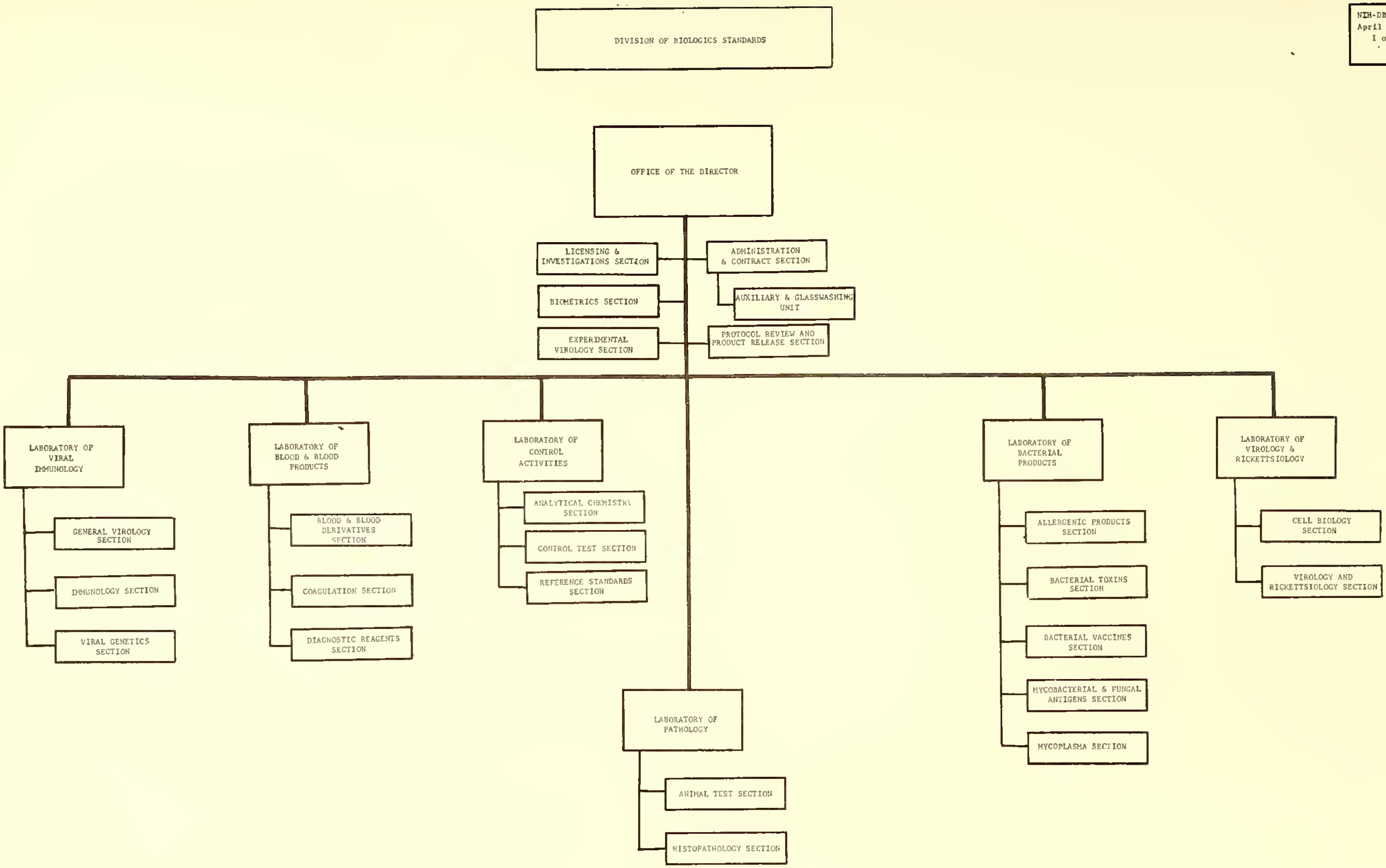
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BIOMETRICS SECTION
EXPERIMENTAL VIROLOGY SECTION
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CELL BIOLOGY SECTION
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Approved:

Date: 5th [illegible] 1971

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Annual Report
# of the
Laboratory of Bacterial Products
Division of Biologics Standards
July 1, 1970 through June 30, 1971

The Laboratory of Bacterial Products had an administrative change resulting from the retirement of Dr. M. Pittman as Chief and the appointment of Dr. H. Baer as the new Chief. No major changes occurred in either the research or collaborative programs of the Laboratory.

The research program of LBP involves a variety of projects designed to investigate basic mechanisms of allergy and infectious diseases as well as applied projects designed to develop new or improved standards or testing procedures for products subject to license under the Public Health Service Act.

In addition, the staff spends much time in review of license applications, amendments, labels and IND reviews as well as travel to inspect licensed establishments and those establishments seeking a license.

Research Activities:

Allergenic Extracts. A potency assay for short ragweed extract has now been reduced to practical form based on a radialimmunodiffusion assay for antigen E. Manufacturers of allergenic extracts are now engaged in a collaborative project with DBS, and it appears that the first potency assay for an allergenic extract will be available within the year. This is an excellent example of a basic research project leading to an applied test applicable to DBS activities. Work on grass pollen extracts is still in progress in collaboration with a group at Johns Hopkins University, and it appears that a similar test can be devised for these extracts based on the skin-reactive group I allergen.

New and more sensitive techniques for the assay of allergens are needed and studies will have to be carried out for this purpose.

The studies on the relationship of mite antigens to the allergenic activity of house dust extracts are yielding interesting results. Many, but not all, house dust extracts contain mite antigens, but, curiously, the antigens seem to be either of different kinds or in different molecular forms since different immunologic tests are required for their detection. Consequently quantitation is not possible at present.

Poison Ivy and Poison Oak: It has been advocated at various times that poison ivy and oak extracts be administered by varying routes to effect desensitization, therefore much effort was expended on studying the physiological and immunological effect of administering pentadecylcatechol and other sensitizers by varying routes. These materials are rapidly absorbed from the skin and appear in the blood stream, excreta and most organs. Whether or not

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sensitivity or tolerance results is a complex matter and exact conditions leading to one or the other cannot yet be accurately defined. This study is continuing.

Cholera and Cholera Antigens: Since current cholera vaccines do not provide long-lasting immunity, a search has been made for other than a whole organism vaccine. Cholera toxoid, prepared from cholera toxin, in preliminary studies appears to hold promise as a new immunizing agent. It is relatively easy to purify the toxin and, therefore, a fairly pure toxoid can be made. The first studies in humans indicated that the toxoid gave many local reactions. Experiments in guinea pigs and monkeys show that these local reactions can be reproduced and may possibly result from the presence of cholera toxin in the toxoid. The presence of a small amount of toxin indicates that the toxin-toxoid reaction is reversible.

Typhoid Vaccine: The role of the various antigens of *Salmonella typhosa* in inducing immunity in humans is still an unsettled matter; the role of Vi antigen is possibly the most perplexing. A typhoid vaccine after the enzymatic removal of the Vi antigen was shown to confer specific immunity in mice to *S. typhosa* which was transferable with serum. Therefore, there are mouse protective factors in this vaccine which are not Vi antigen. The importance, if any, of Vi antigen in immunity in humans is still uncertain. As an aid in this study, a new method of isolating and measuring Vi antigen was developed; the product is free of somatic antigens.

Pseudomonas Vaccine: Pseudomonas vaccine, a mixture of soluble antigens from seven different serologic types, is a product proposed for use in certain selected individuals, i.e., those suffering severe burns. It has been demonstrated that the product is largely, if not exclusively, endotoxin. Nevertheless, it can confer protection to mice challenged with living organisms and this can form the basis for a potency assay if this vaccine should become a licensed product.

Mycoplasma: It has been shown that many cell lines are contaminated with mycoplasmas. Studies with such cell lines have now shown that interferon production may be reduced while virus yields are increased. A new simian species of mycoplasma has been isolated as well as some new species from bovine plasma which seems to be frequently contaminated with these organisms.

Tetanus Studies: Tetanus toxin appears to be a mixture containing at least a neurotoxin and a hemolytic toxin, the latter usually referred to as tetanolysin. The tetanolysin can destroy not only red cells but also platelets. The physiologic effects resulting from cell lysis are under investigation.

Pertussis Immunoglobulins: A study was carried out using the mouse lung infection model to evaluate the role of various immunoglobulins in protection against infection by *B. pertussis*. Tracheobronchial washing and serum revealed a significant rise in IgA capable of binding to *B. pertussis* organisms, which correlated with clearance of the infection. This suggests that IgA may be an essential factor in pertussis immunity.

Tuberculins: There are many species of mycobacteria that can induce sensitivity in humans. The taxonomy of these organisms is not well established nor is it yet possible to clearly distinguish the causative agent for specific sensitivity induced in human beings by natural infection. Immunoelectrophoretic studies of culture filtrates showed that these organisms share many antigens. However, skin tests using culture filtrates as antigens in homologously and heterologously sensitized guinea pigs seems to provide a degree of specificity adequate to differentiate the sensitizing antigens. The skin reaction eventually may also prove to be an important aid in improving the taxonomic status, especially of the avium-intracellulare group.

Studies with tuberculoprotein and carbohydrate from a BCG culture filtrate showed that the protein could induce lymphocyte transformation and inhibit the migration of peritoneal macrophages. The polysaccharide, however, could only induce inhibition of macrophage migration. Not only does this provide a striking difference between these two in vitro tests, but may aid in elucidating their mechanisms.

Control Activities:

Potency and Safety Tests

The Laboratory of Bacterial Products performed tests on and/or reviewed protocols as follows:

Tests and protocol reviews

Type of product	Purpose	Type of test Potency	 Safety Toxicity	Protocols reviewed
Pertussis Vaccine	Official*	141	100	131
	Special	12	10	
Cholera Vaccine				8
Pertussis Immune Globulin (Human)	Official	8		7
Haemophilus influenzae typing serum	Official	2		7
BCG Vaccine	Official	32**		34
			Mycoplasma only	
Measles Vaccine	Official		6	105
Mumps Vaccine	Official		6	25
Poliovirus Vaccine	Official		13	32
Rubella Vaccine	Official		3	46
Intramural			23	
Total		195	161	395

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* Tests of samples of lots for release, inspection or license application.
**Viability counts.

Activities requiring travel status

Type of activity	Personnel	Trips	Locations
Annual inspections	10	16	32
Contracts	4		8

License applications and amendments and notices for investigation of new drugs (IND)

Category	Number	Person participation
License	33	58
IND	57	100

Other Activities and Honors:

Dr. Margaret Pittman.	Cholera Advisory Committee, NIAID, Liaison Member
	Committee on Immunization, Armed Forces Epidemiological Board, Associate Member
	U.S. Pharmacopeia, Member of Advisory Panel on Biological Indicators
	Guest Lecturer, Howard University
	American Society for Microbiologists, Member of Archives Committee and International Acitivities Committee.
	NIAID, Member of Ad Hoc Advisory Committees on Pneumococcus, Meningococcus and *Haemophilus influenzae* Type b Vaccines
Dr. Harold Baer.	Committee on Standardization of Allergens, American College of Allergy
	Committee on Specific Therapy, American Academy of Allergy
	Diagnostic Skin Test Committee of the American Thoracic Society
	U.S. Civil Service Commission, Panel Member of Interagency Board of U.S. Civil Service Examiners
	NIAID, Research Reference, Reagents Branch, Ad Hoc Committee for Antigen E

Dr. S. D. Chaparas.	NIH Graduate Program, Faculty Chairman, Microbiology and Immunology
	International Working Group of Mycobacterial Taxonomy, Working Member
	American Society for Microbiology, Washington, D.C. Branch, President
	Trudeau Institute, Inc., Member Advisory Committee
	International Association of Microbiological Societies, International Committee on Systematic Bacteriology, Subcommittee on Mycobacterium, Corresponding Member
	U.S.-Japan Medical Cooperative Program, Panel on Tuberculosis, Antigen Study Committee, Member
	American Thoracic Society, Scientific Assembly on Microbiology and Immunology, Membership Chairman
Dr. Michael F. Barile.	Chairman, Section on Mycoplasma, American Society for Microbiology
	NIH Graduate Program, Instructor in Microbiology
	Guest Lecturer, University of Maryland
Dr. John C. Feeley.	NIAID, Member of Cholera Advisory Committee
	U.S.-Japan Cooperative Medical Science Program, Cholera Panel Member
	WHO Expert Panel on Bacterial Diseases (Cholera), Member 1967-72
	University of North Carolina, School of Public Health, Adjunct Associate Professor
	NIAID, Member of Ad Hoc Pneumococcal Vaccine Advisory Committee
	Consultant on Cholera Vaccine, Agency for International Development
	Project Officer for two NIAID Contracts on cholera toxin or toxoid (PH-43-67-1428 and NIH-70-2102)

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Dr. C. R. Manclark.	American Society for Microbiology, Washington, D.C. Branch, Education Committee Chairman International Committee on Bacterial Nomenclature, Subcommittee on Taxonomy of Vibrios, Member NIH Graduate Program, Faculty Member, Medical Bacteriology
Dr. Carl E. Miller.	Member, Board of Directors, Association for Gnotobiotics Chairman, School Science Advisory Committee, National Capital Area Branch, American Association for Laboratory Animal Science Member, Animal Technician Certification Board, American Association for Laboratory Animal Science

Staff members have participated in national and international symposia, conferences, workshops, delivered lectures, and judged at science fairs.

Serial No. DBS 102
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunology of the Delayed Contact Sensitivity Induced by Catechols Related to and Contained in Poison Ivy Extracts

Previous Serial Number: Same

Principal Investigators: Harold Baer, Ph. D.
Mitchell H. Friedlaender, M.D.

Other Investigators: C. J. Maloney, Ph. D. (OD)

Cooperating Units: Office of the Director

Man Years

Total:	2.5
Professional:	1.25
Other:	1.25

Project Description:

Objectives: (1) To examine the composition and to establish a method for the standardization of poison ivy extracts and related plants such as poison oak and sumac. (2) To determine the relationship of structure of catechols to their effectiveness in inducing delayed contact sensitivity and immune tolerance, and (3) to study the mechanism of delayed contact sensitivity and immune tolerance.

Methods Employed: (1) Guinea pigs sensitized to various synthetic catechols were used to evaluate the relationship of catechol structure to immunologic specificity and to their ability to induce immune tolerance and delayed contact sensitivity. (2) Sensitizing chemicals were placed on guinea pig skin under varying conditions to determine the rate of reaction with or removal from the skin. (3) Sensitizing chemicals were put onto the skin of guinea pigs in such manner as to study the importance of draining lymphatics and blood vessels on the induction of sensitivity and tolerance. (4) Autologous skin transplants in guinea pigs were used to determine the importance of simple chemicals in free and conjugated forms in the induction of delayed contact sensitivity and immunologic tolerance.

Major Findings: (1) Chemicals that are nonsensitizers do not induce immune tolerance to chemically related sensitizers. (2) Chemicals that are very poor sensitizers may induce intense tolerance to chemically related

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sensitizers. (3) Sensitization and tolerance depend on the presence of draining lymphatics.

Significance to Bio-Medical Research and the Program of the Division: These studies are providing information on the mechanism of sensitization and the induction of immune tolerance. They may point the way toward using chemicals that are poor sensitizers, and hence cause few reactions, to induce tolerance or to rapidly and safely desensitize sensitive humans.

Proposed Course of Project: The project will be continued until all major objectives have been reached.

Honors and Awards: None

Publications:

Godfrey, H. P., Baer, H., and Watkins, Roy C. Delayed hypersensitivity to catechols. V. Absorption and distribution of substances related to poison ivy extracts and their relation to the induction of sensitization and tolerance. J. Immunol. 106:91-102, 1971.

Godfrey, H. P. and Baer, Harold. The effect of physical and chemical properties of the sensitizing substance on the induction and elicitation of delayed contact sensitivity. J. Immunol. 106:431-441, 1971.

Godfrey, H. P., and Baer, Harold. Antibodies produced after immunization with substituted *Meta*-Dinitrobenzenes. Production of γ_1 and γ_2 classes of antibody including complement-dependent antibodies. J. Immunol. (In press)

Serial No. DBS 119
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Allergenic Extracts

Previous Serial Number: Same

Principal Investigators: Harold Baer, Ph. D.

Other Investigators: Philip Norman, M.D., Johns Hopkins
Lawrence Lichtenstein, M.D., Johns Hopkins

Cooperating Units: Johns Hopkins Medical School

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: (1) To measure the presence, stability, and quantity of antigen E in commercial ragweed extracts. (2) To immunize rabbits with extracts of various pollens to produce antisera capable of estimating the quantity of extract in mixed commercial extracts. (3) To study extracts of pollens from various crop years to determine whether the antigen content is affected by crop year or by storage of pollen. (4) To study the antigens of grass pollen extracts.

Methods Employed: (1) Gel diffusion for the quantitative measurement of the concentration of antigen E in commercial extracts. (2) Gel diffusion studies on grass pollen extracts in an attempt to correlate gel diffusion bands with allergenicity in humans. (3) Skin tests in sensitive humans and histamine-release tests to determine the relationship of laboratory assayed antigens to human sensitivity (NIH-69-2010). (4) Preparation of extracts of short ragweed pollens from various crop years to determine the effect of pollen age on antigen E content of extracts.

Major Findings: Radialimmunodiffusion has proven to be a useful method for measuring the antigen E content of commercially prepared short ragweed extracts. Cooperative studies between DBS and licensed manufacturers indicate that the method will probably be useful for the routine potency assay of such extracts. The method using appropriate reagents also appears to have a potential for assay of the potency of certain grass pollen extracts.

Studies on grass pollen extracts are preliminary but indicate that PNU again will be unrelated to the human potency of these extracts.

Significance to Bio-Medical Research and the Program of the Division: The correlation of antigen E with potency now makes it possible to have a potency assay for short ragweed extracts. It will be possible to have extracts with more uniform potency and with a meaningful dating period.

Extracts prepared at DBS from many lots of pollen revealed that there was a wide variation in the antigen E content and hence variation in the potency of these extracts. Therefore, procedures based on weight of pollen to volume of extracting fluid cannot yield extracts of uniform potency.

The human reactivities of grass pollen extracts are unrelated to the usual criteria of laboratory assayed potency and, therefore, a laboratory assay will await the finding of one or more specific antigens.

Proposed Course of Project: (1) To formulate a practical assay for ragweed antigen E. (2) To extend the studies to other pollens and their extracts.

Honors and Awards: None

Publications:

Baer, H., Godfrey, H., Maloney, C. J., Norman, P. S., and Lichtenstein, L. M.: The potency and antigen E content of commercially prepared ragweed extracts. J. Allergy 45:347-354, 1970.

Serial No. DBS 134
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Methods of Skin Testing Humans for Allergy

Previous Serial Number: Same

Principal Investigators: Harold Baer. Ph. D.
William B. Grater, M.D. (CSACA)

Other Investigators: B. A. Berman, M.D.
R. Hale, M.D.
M. Scherr, M.D.
W. Swinney, M.D.
C. J. Maloney, Ph. D. (OD)

Cooperating Units: Office of the Director
Committee on Standardization of the American College of Allergy

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description: This project is now complete.

Publications:

Scherr, Merle S., Grater, William C., Baer, Harold, Berman, Bernard A., Center, J. George and Hale, Ralph: Report of the committee on standardization: I. A method of evaluating skin test response. _Ann. of Allergy_ 29:30-34, 1971.

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Serial No. DBS 159
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunology of the Marmoset, *Saguinus mystax*

Previous Serial Number: Same

Principal Investigators: Harold Baer, Ph. D.
Douglas Lorenz, Ph. D. (LP)

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description: This project is now complete

Publications:

Baer, H. and Lorenz, D.: The induction of immediate and delayed sensitivity in the marmoset. *Proc. Soc. Exptl. Biol. and Med.* 134:410-412, 1970.

Serial No. DBS 206
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the House Dust Mites *D. farinae* and *D. pteronyssinus*

Previous Serial Number: None

Principal Investigator: Harold Baer, Ph. D.
Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: (1) To study the antigens of mites. (2) To determine whether commercial house dust extracts contain mite antigens and whether the presence of these antigens can be correlated with the reactivity of these extracts when injected into the skin of allergic humans.

Methods Employed: Mites were obtained from Ohio State University where they are being cultured under a DBS contract. Extracts were prepared and the antigens studied by immunization of rabbits to produce precipitating antisera. Antigens were also sought by the use of PCA and inhibition of PCA tests.

Major Findings: (1) There are many antigens in both *D. farinae* and *D. pteronyssinus*. (2) These species are antigenically different but share some antigens. (3) Some, but not all, commercially prepared house dust extracts contain mite antigens.

Significance to Bio-Medical Research and the Program of the Division: Although house dust extracts have been investigated for many years, no unique house dust allergen has been uncovered. If mites prove to be the unique, or even the major source of house dust allergen, it might become possible to standardize house dust extracts or to supplant them with one or more specific extracts.

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Proposed Course of Project: To continue until the main objectives have been reached.

Honors and Awards: None

Publications: None

Serial No. DBS 12
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of Tetanus Toxoids for Prevention of Neonatal Tetanus

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Michael F. Barile, Ph. D.
Margaret Pittman, Ph. D.

Other Investigators: Frank Schofield, M.D., New Guinea
Robert MacLennan, M.D., New Guinea
A. Kelly, New Guinea
Clifford J. Maloney, Ph. D. (OD)

Cooperating Units: Department of Public Health, Territory of Papua and New Guinea
Office of the Director, Biometrics Section

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: The objectives have been completed and included studies on the (1) persistence of tetanus antitoxin in New Guinea women immunized since 1962 with adjuvant and plain toxoids; (2) the booster response in these women after a plain or adsorbed toxoid; (3) the antitotxin response in guinea pigs by the immunization schedules used in New Guinea; (4) the tetanus antitoxin titers obtained by the hemagglutination and mouse neutralization procedures.

Methods Employed: Immunization of guinea pigs and titration (1) of antitoxin responses of guinea pigs by mouse neutralization test and (2) of women by both neutralization and hemagglutination tests.

Major Findings: The findings were reported in three manuscripts.

Significance to Bio-Medical Research and the Program of the Division: Information has been obtained on the duration of the antitoxin level which protects against neonatal tetanus as influenced by adjuvants. The field results provide information on human antitoxin response to the laboratory assayed potency of tetanus toxoids. The findings are applicable to the potency and safety specifications for tetanus toxoid.

Proposed Course of Project: (1) The main objectives have been completed, and the findings published. (2) The related studies to determine cause of untoward reactivity of oil adjuvants (Proj. No. 15, p.) will be continued.

Honors and Awards: None

Publications:

Hardegree, M. C., Barile, M. F., Pittman, M., Schofield, F. D., MacLennan, R., and Kelly, A. Immunization against neonatal tetanus in New Guinea. 2. Duration of primary antitoxin responses to adjuvant tetanus toxoids and comparison of booster responses to adjuvant and plain toxoids. Bull. Wld Hlth Org. 43:439-451, 1970.

Barile, M. F., Hardegree, M. C., and Pittman, M. Immunization against neonatal tetanus in New Guinea. 3. The toxin-neutralization test and the response of guinea pigs to the toxoids as used in the immunization schedules in New Guinea. Bull. Wld Hlth Org. 43:453-459, 1970.

Hardegree, M. C., Barile, M. F., Pittman, M., Maloney, C. J., Schofield, F. and MacLennan, R. Immunization against neonatal tetanus in New Guinea. 4. Comparison of tetanus antitoxin titres obtained by hemagglutination and toxin neutralization in mice. Bull. Wld Hlth Org. 43:461-468, 1970.

Serial No. DBS 13
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A Study of the Antigenic Substances of *Clostridium tetani*

Previous Serial Number: Same

Principal Investigator: M. Carolyn Hardegree, M.D.

Other Investigator: Mrs. Claire Cox, M.S.

Cooperating Units: None

Man Years

Total:	1.1
Professional:	0.7
Other:	0.4

Project Description:

Objectives: (1) To determine and separate the antigens present in culture filtrates of *Cl. tetani*. (2) To characterize the *in vivo* and *in vitro* activities of these substances. (3) To determine their relationship to optimal anti-tetanus immunization.

Methods Employed: Column chromatography, zone electrophoresis, gel diffusion, immunoelectrophoresis, enzyme assays, plasma hemoglobin determination, thin layer chromatography, gas chromatography, animal bioassay, animal immunization.

Major Findings: Tetanolysin destroys platelets as well as erythrocytes.

Significance to Bio-Medical Research and the Program of the Division: It is expected that the investigation will contribute to an understanding of the role of antibodies against *Cl. tetani* antigens other than the neurotoxin. These antibodies may be necessary for the prophylaxis and the treatment of tetanus. Findings will provide fundamental information for the control of tetanus toxoid and tetanus immune serum globulin.

Proposed Course of Project: Studies will be continued on (1) the isolation and recovery of components of the culture filtrates, (2) the continued characterization of the *in vivo* and *in vitro* effects of the hemolysin, (3) the nature of the inactivation of the hemolysin by ganglioside and ganglioside

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complexes, and (4) on the relationship of the peptidase to the neurotoxin. Studies will be initiated to evaluate the effects of *Cl. tetani* filtrates on blood coagulation and on the sympathetic nervous system.

Honors and Awards: None

Publications:

Hardegree, M. C., Palmer, A. E. and Duffin, N. Tetanolysin: *In vivo* effects in animals. *J. Infect. Dis.* 123:51-60, 1971.

Serial No. DBS 14
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Reference Preparations for Tetanus Toxoids

Previous Serial Number: Same

Principal Investigators: Margaret Pittman, Ph. D.
Michael F. Barile, Ph. D.
M. Carolyn Hardegree, M.D.
Robert W. Kolb, M.S. (OD)
Edward B. Seligmann, Jr., Ph. D. (LCA)

Other Investigators: None

Cooperating Unit: Laboratory of Control Activities

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: The objectives have been completed and the project terminated.

Significance to Bio-Medical Research and the Program of the Division: The results of this study are related to investigations directed towards the establishment of standards for quantitative potency assays of tetanus toxoids and the relation of unitage to the initial human response and to the persistence of protective levels of antitoxin.

Proposed Course of Project: Although this project has been terminated, the results will be applied to the development of U. S. requirements for quantitative potency assays of tetanus toxoids.

Honors and Awards: None

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Publications:

Pittman, M., Kolb, R. W., Barile, M. F., Hardegree, M. C., Seligmann, E. B., Jr., MacLennan, R. and Schofield, F. D. Immunization against Neonatal Tetanus in New Guinea. 5. Laboratory Assayed Potency of Tetanus Toxoids and Relationship to Human Antitoxin Response. Bull. Wld Hlth Org. 43:469-478, 1970.

Serial No. DBS 15
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of Toxicity of Oil Emulsified Antigens

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Michael F. Barile, Ph. D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To determine factors responsible for sterile abscesses in recipients of oil emulsified biologics. To examine the use of cell cultures for the evaluation of oil adjuvant component toxicity and to evaluate the mechanisms of this toxicity.

Findings: DMSO enhances the toxicity of Arlacel A and fatty acids in six cell culture lines and in mice. The cell culture assay can be used for the evaluation of the toxicity of vegetable and mineral oils.

Significance to Bio-Medical Research and the Program of the Division: These studies provide information on the reactivity of components of oil emulsified antigens and may be useful in developing specifications for oil emulsified products should the use of oil adjuvants be approved.

Proposed Course of Project: To continue to evaluate various in vivo procedures for the evaluation of the toxicity of the components of oil adjuvants and to continue to evaluate the relationship of the FFA in the emulsifier, Arlacel A, to reactivity. To use cell cultures to evaluate the toxicity of other adjuvant components. Findings are being prepared for publication.

Honors and Awards: None

Publications: None

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Serial No. DBS 181
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of the Role of Specific Immunoglobulins in Pertussis Immunity

Previous Serial Number: Same

Principal Investigator: Bernard D. Geller, Ph. D., M.D.

Other Investigators: Margaret Pittman, Ph. D.
Linda A. Smallwood

Cooperating Units: None

Man Years

Total:	2.5
Professional:	1.7
Other:	0.8

Project Description:

Objectives: (1) To find the basis of protection afforded by pertussis vaccine against whooping cough, a local respiratory infection. (2) To determine the role of local immunoglobulins using the respiratory *Bordetella pertussis* infection mouse model. (3) To determine the effect of Pertussis Immune Globulin (Human) on mouse lung infection and the development of histamine sensitization.

Methods Employed: (1) Fractionation of Pertussis Immune Globulin (Human), human hyperimmune plasma and mouse hyperimmune serum by column chromatography on Sephadex, Sepharose, and DEAE-cellulose. (2) Reconstitution to original concentration by pervaporation in collodion bags. (3) Quantitation by immunoelectrophoresis, immunoelectrodiffusion, radial diffusion in agar and cellulose acetate and simple double diffusion in order to measure the amounts of IgG, IgA and IgM in starting materials and fractions. (4) Mouse tests to determine (a) passive protective activity of whole Pertussis Immune Globulin (Human) against respiratory infection and histamine sensitization, (b) the presence of secretory and other immunoglobulins in the respiratory tract following passive protection by use of fluorescent microscopy, (c) amount of local antibody produced in respiratory infections measured by use of naso-tracheal washes, and (d) the relative activity of specific immunoglobulins in hyperimmune plasma *vs* globulin to bind to *B. pertussis* organisms as measured by indirect fluorescence microscopy.

Major Findings: Part I. Human globulins. Results show that immunoglobulin subclasses in plasma used for production of Cohn Fraction II Globulin are within normal levels, while the final product showed from 8 to 10 times concentration of IgG but not of IgA or IgM. The quantitation of their relative binding capabilities to B. pertussis organisms is currently under investigation. These globulins appear to participate in clearance of lung infection and can be shown binding to B. pertussis organisms in mouse lung within 3 hours of simultaneous administration of bacteria intranasally and globulin intraperitoneally.

Part II. Mouse infection study. Using the method we developed for recovery, we found nasotracheal wash from the mouse to contain IgA, IgG_1, IgG_{2A} and IgG_{2B} but not IgM. Serum IgA increased greatly in level late in the infection, apparently after the organisms were eliminated from the respiratory passages.

Significance to Bio-Medical Research and the Program of the Division: These studies provide (1) information on the levels and activities of specific immunoglobulins in Pertussis Immune Globulin (Human) and antipertussis serum and may give insight into the efficacy of such products, and (2) basic information on the role of immunoglobulins in protection against the mouse respiratory tract infection with B. pertussis which may be applicable to an understanding of the efficacy of pertussis vaccine.

Proposed Course of Project: To continue as outlined above.

Honors and Awards:

Geller, Bernard D. and Pittman, Margaret. Mouse Immunoglobulin Response to Lung Infection with Bordetella pertussis. (Abstract) For presentation at American Thoracic Society Annual Meeting, Los Angeles, May 1971.

Publications: None

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Serial No. DBS 204
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Potency Assay of Tetanus Toxoids in Mice

Previous Serial Number: None

Principal Investigator: M. Carolyn Hardegree, M.D.

Other Investigator: Margaret Pittman, Ph. D.

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: To evaluate the usefulness of the mouse for the assay of the potency, in International Units, of tetanus toxoids.

Methods Employed: Several strains of mice were vaccinated with graded doses of tetanus toxoids and their responses were determined by challenge with tetanus toxin. The unitage of the toxoids was calculated using the International Standards for Tetanus Toxoid, Adsorbed and Plain.

Major Findings: (1) The unitage calculated for toxoid C_2, adsorbed, appears to be dependent on the mouse strain used for assay. (2) C3H mice could not be immunized at doses adequate to protect four other strains of mice. (3) Preliminary data suggests that NIH mice are suitable for the assay of plain as well as adsorbed toxoids.

Significance to Bio-Medical Research and the Program of the Division: The use of International Units (I.U.) for describing the potency of tetanus toxoids provides a quantitative basis for the comparison of toxoids. The I.U. of the toxoid may be related to the duration of the antitoxin response in man. A mouse assay would be more economical and less space consuming than a guinea pig test if the potency determined in mice could be shown to be relevant to the potency in man. This data should provide information on which strains of mice may be suitable for the assay.

Proposed Course of Project: (1) To assay more toxoids in mice to see if there is a statistically significant difference in the unitage of adsorbed toxoids as determined in different strains of mice. (2) To evaluate the effect of the aluminum content of toxoids, derived from the same parent, on the unitage. (3) To further evaluate the use of mice for the potency assay of fluid toxoids. (4) To examine the feasibility of applying this assay as a required test for all commercial toxoids.

Honors and Awards: None

Publications: None

Serial No. DBS 205
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
July 1, 1970 through June 30, 1971

Project Title: A Study of Biologics as Interferon Inducers

Previous Serial Number: None

Principal Investigators: M. Carolyn Hardegree, M.D.
Stanley Singer, M.D.

Other Investigator: Peter J. Gerone, Sc. D.

Cooperating Units: Laboratory of Pathology
Virus and Rickettsia Division, Ft. Detrick

Man Years

Total	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: (1) To determine whether or not bacterial vaccines with no U.S. standard of potency are capable of inducing interferon; (2) To determine whether or not allergenic extracts are capable of inducing interferon.

Methods Employed: (1) Injection of mice by various routes with biologics; (2) Determination of interferon titers in pooled mouse sera using standard virus yield reduction method in primary mouse embryo fibroblast cell cultures; (3) Determination of antiviral activity in mice challenged with a dose of virus, either by IP injection or by aerosol.

Major Findings: (1) High levels of circulating interferon were induced by certain of the bacterial vaccines and allergenic extracts; (2) Interferon was induced when either the IV or SC route was used; (3) The time of appearance and the duration of the interferon production were similar to those reported for endotoxin; (4) Mice injected with a bacterial vaccine or an allergenic extract of house dust IV were protected against a lethal virus challenge.

Significance to Bio-Medical Research and the Program of the Division: Information has been obtained which shows that certain licensed biologics have the capacity to induce interferon in mice and to protect mice against a viral challenge under certain experimental conditions. It is possible that this activity may be associated with the clinical effectiveness of this type of product in man.

Proposed Course of Project: (1) To continue to evaluate the effect of these products on challenge by aerosol with respiratory viruses; (2) To evaluate the effect of repeated doses on interferon titers and viral resistance; (3) To determine interferon titers in man following allergenic extracts and/or bacterial vaccines; (4) To evaluate other extracts and bacterial products for interferon inducing capacity.

Honors and Awards: None

Publications:

Singer, S. H. and Hardegree, M. C.: Induction of interferon by bacterial vaccines and allergenic extracts. J. Allergy (In press).

Serial No. DBS 22
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Cholera and Cholera Vaccines

Previous Serial Number: Same

Principal Investigator: John C. Feeley, Ph. D.

Other Investigators: Carl E. Miller, D.V.M.
Meade A. Doyle
Robert S. Northrup, M.D. (NIAID)
Margaret Pittman, Ph. D.

Cooperating Units: NIAID Cholera Advisory Committee
U.S.-Japan Cooperative Medical Science Program
Pakistan-SEATO Cholera Research Laboratory
Laboratory of Pathology

Man Years

Total:	2.2
Professional:	2.0
Other:	0.2

Project Description:

Objectives: (1) Establishment of a meaningful standard of potency for cholera vaccine. (2) Examination of cholera toxoid as an immunizing agent against cholera.

Methods Employed: Various purified toxins and toxoids prepared under NIAID contract were investigated. Assays for cholera toxin were based on the skin permeability factor (PF) assay. Toxoids were examined by gel diffusion and flocculation tests and assayed for innocuity by injection of mice (intraperitoneally and intravenously) and guinea pigs (subcutaneously). Residual somatic antigen content of purified cholera toxoids was estimated by mouse protection tests using live vibrio challenge. Various sera from rabbits, dogs, monkeys, and human volunteers immunized with these toxoids were assayed by agglutination and vibriocidal tests for antibodies against somatic antigens, and by hemagglutination and PF neutralization tests for antitoxin content. Antibody responses of rabbits to fluid and aluminum-hydroxide adsorbed toxoids were evaluated.

Major Findings: Subproject 1: Standardization of Cholera Vaccine. No additional studies on standardization of cholera vaccine have been conducted. Information accumulated has formed the basis for preparation of a draft of Additional Standards: Cholera Vaccine, which has been submitted to the Office of the Director, DBS. A publication showing the correlation of vaccine potency as determined by the mouse protection test with antigenicity and protective activity in man is listed below. No further studies of this subject are contemplated.

Subproject 2: Cholera Toxoid Studies: Cholera toxoid prepared under NIAID contract for initial clinical studies in man has been examined. This toxoid was low in somatic antigen content as judged by active mouse protection tests against live vibrio challenge and gave a single band in gel diffusion and immunoelectrophoretic experiments. It was free from detectable residual toxin and innocuous in large doses in mice, guinea pigs, and dogs. Rabbits immunized with toxoid or parent toxin gave good antitoxin responses with only minimal rises in vibriocidal antibody.

In initial clinical studies conducted at the University of Texas, the toxoid was found to produce mild to moderate local edema appearing at the injection site 48-72 hours after injection of doses ranging from 2-13 micrograms. Edema persisted for 7-14 days. With doses of 25 micrograms, unacceptably severe local reactions resulted. These findings suggested possible in vivo reversion to toxicity at the injection site. Following this experience, it was found that detectable reversion to toxicity occurred in vitro in toxoid incubated at 25°C or 37°C, but not at 4°C. Subsequently, monkeys immunized with 50 micrograms developed reactions similar to those seen in humans, even through this had not been observed in other animal species. On re-immunization four weeks later (after antitoxin was present) no local reactions were produced, an observation consistent with the likelihood that these reactions were due to toxin reversion. This experience indicates the need for further studies to develop a stable toxoid satisfactory for field trial, and these are now in progress in collaboration with the contractor.

Preliminary experiments on the production of an aluminum hydroxide adsorbed "adjuvanted" toxoid were carried out. Previous difficulties in adsorbing toxoid to aluminum hydroxide were overcome by use of a special preparation of high adsorptive capacity. In spite of apparently satisfactory adsorption, no adjuvant effect was observed in rabbits immunized with adsorbed toxoid as compared to fluid toxoid. Further studies employing different adsorption conditions and also aluminum phosphate are in progress or are planned.

The hemagglutination assay for cholera antitoxin developed in this laboratory has been employed in the assay of sera from dogs and humans immunized with toxoid. The hemagglutination test, which has generally shown an excellent correlation with in vivo antitoxin titrations on human and rabbit sera, proved unsatisfactory with dog sera. Weak or negative hemagglutination reactions were obtained on many sera which were demonstrated to contain antitoxin by in vivo neutralization tests. A few sera from toxoid-immunized human volunteers showed unusually high hemagglutination titers as compared with toxin-neutralizing ability, suggesting the possibility of antibody (perhaps IgM) against toxin antigen which lacked toxin neutralizing ability. Further study of this phenomenon employing 2-mercaptoethanol treatment to inactivate IgM and study of isolated immunoglobulin fractions is planned.

Subproject 3: Characterization of strains of Vibrio cholerae: No new strains of V. cholerae were studied this year. This has become an incidental activity and is terminated as a subproject.

Significance to Bio-Medical Research and the Program of the Division: Presently available cholera vaccines are now known to be capable only of limited protective activity (30-80%) for a period of only a few months. Additional Standards for this product will hopefully now be published for inclusion in Regulations.

The current studies on cholera toxoid are part of the NIH effort to develop a more effective immunizing agent against this disease that will offer a significant degree of hopefully long-term protection. Initial plans called for a field trial to be carried out in East Pakistan in the Fall of 1971, but this has been postponed due to the problems with local reactions. Hopefully, this will be possible by next year. Experience gained in participation in this effort will be vital to establishment of standards for cholera toxoid.

Proposed Course of the Project: To follow through as indicated above.

Honors and Awards: None

Publications:

Gangarosa, E. J., DeWitt, W. E., Feeley, J. C., and Adams, M. R.: Significance of vibriocidal antibodies with regard to cholera immunity. J. Infect. Dis. 121 (Suppl.):S36-S43, 1970. (Reported as In Press" in 1970)

Hochstein, H. D., Feeley, J. C., and DeWitt, W. E.: Titration of cholera antitoxin in human sera by microhemagglutination using formalinized erythrocytes. Applied Microbiol. 19:742-745, 1970. (Reported as "In Press" in 1970)

Mosley, W. H., Feeley, J. C., and Pittman, M.: The interrelationships of serological responses in humans, and the active mouse protection test, to cholera vaccine effectiveness. Symposia Series in Immunobiological Standardization, Basel, S. Karger, 15:185-196, 1971 (Reported as "In Press" in 1970)

Mosley, W. H., Woodward, W. E., Aziz, K.M.A., Rahman, A.S.M.M., Chowdhury, A.K.M.A., Ahmed, A., and Feeley, J. C.: The 1968-69 cholera vaccine field trial in rural East Pakistan. Effectiveness of monovalent Ogawa and Inaba vaccines and a purified Inaba antigen with comparative results of serological and animal protection tests. J. Infect. Dis. 121 (Suppl): S1-S9, 1970. (Reported as "In Press" in 1970)

Spaun, J., Feeley, J. C., Smith, H. L., and Thomas, A. K.: Cholera O group I agglutinating serum. Bull. Wld Hlth Org. 42:818-819, 1970.

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Serial No. DBS 116
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Typhoid Vaccines

Previous Serial Number: Same

Principal Investigator: K. H. Wong, Ph. D.

Other Investigators: John C. Feeley, Ph. D.
Margaret Pittman, Ph. D.

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To establish a valid potency test for typhoid vaccines which will accurately reflect their efficacy for man. Immediate objectives were: (1) to determine the role of Vi and other protective factor(s) in the U.S. standard mouse protection assay, (2) to develop a quantitative method for measuring Vi antigen and determining the effect of varying Vi content in vaccines on potency estimation, and (3) to study immune response to typhoid antigen at the cellular level.

Methods Employed: Enzymatic degradation, chemical extraction, active and passive mouse protection tests, electro-immunodiffusion, fluorescent antibody, and other immunological and serological techniques were used.

Major Findings: (1) Using a Vi degrading enzyme isolated from *Bacillus sphericus*, it was found that protection of *Citrobacter* 5396/38 vaccine against *S. typhosa* strain TY 2 challenge in mice was due to the Vi antigen. The U.S. standard typhoid vaccine 6A and World Health Organization field trial typhoid vaccine L possessed protective factor(s) other than Vi which are absent in the Citrobacter vaccine. The immunity conferred by these factor(s) was specific for the typhoid bacillus and could be transferred passively by immune serum.

Vaccines 6A and L differed with respect to the Vi antigen. The effect of the Vi-degrading enzyme on the potencies of these vaccines varied with the route of immunization. For intraperitoneal vaccination, enzyme treatment caused a decrease in potency of about 47 times in 6A but only about 8 times in L. By subcutaneous immunization, the decrease was 12 and 13 times for 6A and L respectively. Vi antigen was the major protective factor in an assay where intraperitoneal vaccination and challenge were used.

(2) A method was developed for the isolation of Vi antigen from bacterial cells. This involved treatment of bacterial cell extracts with deoxyribonuclease, ribonuclease, and pronase, followed by extration with alcohol at different temperatures and differential precipitation with hexadecyltrimethylammonium bromide.

The Vi antigen isolated by this method was free from somatic antigen, gave one clear precipitin band in double gel diffusion, and at least five to eight times more protective in mice than the other Vi antigen isolated by a procedure involving acid hydrolysis.

This is a relatively simple and mild method and may be suitable for the preparation of Vi antigen in quantity for immunological studies.

(3) A method based on electro-immunodiffusion was established for quantitation of Vi antigen. Sensitivity range was dependent on serum concentration. From 10 to 80 micrograms antigen per ml, the antigen-antibody precipitate length was related arithmetically to the concentration of antigen. Above this range, it was proportional to the logarithm of antigen concentration.

(4) Using the above quantitation method, a good correlation was found between the varying Vi content of typhoid vaccines and their potency as estimated by the DBS mouse protection potency test.

Significance for Bio-Medical Research and the Program of the Division: The Vi antigen is protective for mice but its significance in human immunity is unclear. Determination of the influence of the various protective antigens is important in establishing a potency test which will accurately reflect the prophylactic efficacy of the vaccine in man. Isolation of highly immunogenic Vi antigen may help to determine if the antigen is necessary for human protection.

Proposed Course of Project: Emphasis will be on cellular response to typhoid antigen. Understanding of the basic immune mechanism is essential for the development of a scientifically valid potency assay. Results to date will be published.

Honors and Awards: None

Publications:

Esposito, V. M., Feeley, J. C. and Pittman, M.: Immunological responses of different strains of mice to typhoid vaccine and Vi antigen. International Symposium on Enterobacterial Vaccines, Berne 1969; Symp. Series Immunobil. Stand. Basel/Munchen/New York, Karger, 1971, vol. 15, pp. 137-142.

Serial No. DBS 136
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of Microbially Controlled Animals in Control Testing and Infection

Previous Serial Number: Same

Principal Investigator: Carl E. Miller, D.V.M.

Other Investigators: Kwei-Hay Wong, Ph. D.
John C. Feeley, Ph. D.

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description: Part I. Interplay of Intestinal Microflora on Mouse Susceptibility to Endotoxin.

Objectives: To determine if the resident bacterial flora in the intestine of mice influences their response to vaccine toxicity tests.

Methods Employed: Germfree mice housed in plastic isolators were given various species of intestinal bacteria. The response of these gnotobiotic mice to typhoid vaccine and pertussis vaccine in weight-gain type toxicity tests was compared to the response of conventional mice.

Major Findings: Germfree and limited flora mice have a much higher tolerance to endotoxin than conventional mice. It appears that the gram negative flora of mice may be a major influencing factor in this difference in weight gain in toxicity testing. Various species of gram negative bacteria were screened for their influence on the response of mice to the toxicity test.

Significance to Bio-Medical Research and the Program of the Division: Control of the biological environment of laboratory animals has a pronounced effect on their health and response to stress. The animal may be more susceptible to certain infections but more resistant to other forms of physiological stress. Pertussis and typhoid vaccines that would fail the toxicity test in conventional mice can pass an identical test in gnotobiotic or limited-flora

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mice. With the continued improvement in the quality of laboratory animals, this information may have important implications in control testing of biological products.

Proposed Course of Project: Continuation along the lines outlined above.

Project Description: Part II. Role of the Indigenous Microflora in Resistance of Mice to Infection with *Vibrio cholerae*.

Objectives: To determine which microorganisms are responsible for natural resistance to gastrointestinal infection with *Vibrio cholerae* in mice.

Methods Employed: Plastic isolators were used to control the microbial environment of *Vibrio cholerae* infected mice. These mice were then inoculated with pure cultures of various intestinal bacteria from conventional mice to assess the role of the resident flora in competing with *V. cholerae*.

Major Findings: No single species of bacteria was found that would eliminate cholera vibrios from the intestines of mice. However, a combination of three species, *E. coli*, *Proteus sp.*, and *Streptococcus fecalis* was found to reduce the vibrio population below recoverable levels.

Significance to Bio-Medical Research and Program of the Division: The question of why only some persons in a given environment develop cholera cannot be explained completely on the basis of immunologic considerations. Other factors, such as the indigenous intestinal flora, may play a significant role in competing with the cholera organism. Identification of the microbial factor(s) which permit or prevent colonization in mice may help explain the absence of cholera in exposed individuals.

Proposed Course of Project: This project has been terminated.

Project Description: Part III. Epidemiological Significance of Chronic Carriers of Rough *Vibrio cholerae*.

Objectives: To determine if chronic carriers of rough *Vibrio cholerae* could be a source of cholera from season to season.

Methods Employed: Rough forms of *V. cholerae* isolated from patients' stools were inoculated orally into germfree mice. Reversion to smooth vibrios was detected by destroying the rough forms in mouse stools by treating with guinea pig complement and antirough serum. The serotype of remaining colonies was determined by the slide agglutination test. Colonies in the process of reversion were then serially passed in germfree mice and tested for complete reversion to the smooth, virulent form.

Major Findings: Rough *Vibrio cholerae* organisms are destroyed by adequate amounts of complement and antirough serum. The remaining organisms contain varying amounts of smooth, "O" antigen. Cholera vibrios resembling smooth Ogawa El tor vibrios have been recovered from originally rough organisms.

This strain was common in the Calcutta area at the time these rough vibrios were isolated from chronic carriers.

Significance to Bio-Medical Research and the Program of the Division: Cholera in humans is usually seasonal in the endemic areas. The location of *V. cholerae* in the off-season is not known. Demonstration of revitalization of smooth cholera vibrios from chronic carriers of rough vibrios may provide a partial answer to this question.

Proposed Course of Project: Continuation along the lines outlined above.

Honors and Awards: None

Publications:

Miller, Carl E., and Feeley, John C.: Competitive effects of intestinal microflora on *Vibrio cholerae* in gnotobiotic mice. *Proceedings 1970* Symposium on Cholera, U.S.-Japan Cooperative Medical Science Program, Japan, 1970. (In press)

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Serial No. DBS 182
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Pseudomonas Vaccine

Previous Serial Number: Same

Principal Investigators: John C. Feeley, Ph. D.
Edward B. Seligmann, Jr., Ph. D.

Other Investigators: Jane F. Farber (LCA)
Otis L. Green (LCA)
Kwei-Hay Wong, Ph. D.
Carl E. Miller, D.V.M.
Margaret Pittman, Ph. D.

Cooperating Units: Laboratory of Control Activities

Man Years

Total:	1.8
Professional:	1.2
Other:	0.6

Project Description:

Objectives: To carry out necessary laboratory investigations relative to development of standards for the safety, purity and potency of vaccine made from *Pseudomonas aeruginosa*.

Methods Employed: Active protection tests in mice, toxicity assays in normal and actinomycin-D-treated mice, pyrogen tests in rabbits, and general safety tests in guinea pigs and mice.

Major Findings: Comparisons of the potencies of heptavalent "master" and "working" reference vaccines have been carried out by mouse protection tests challenged with the seven immunotypes of *P. aeruginosa* involved most frequently in human infections. Both reference vaccines appear comparable. Similar comparisons with two additional lots of vaccine indicate potency comparable to the reference vaccines.

The vaccine under evaluation is a heptavalent mixture of purified lipopolysaccharide antigens prepared from the above seven immunotypes. Mouse toxicity tests based either on weight loss of normal mice or lethality in actinomycin-D-treated mice indicate that this product is essentially equivalent in endotoxin activity on a weight basis to purified *Escherichia coli* endotoxin. Pyrogenicity characteristics in rabbits are consistent with this interpretation. The vaccine has passed required General Safety Tests in guinea pigs and mice.

Significance to Bio-Medical Research and the Program of the Division: Clinical trials currently still in progress are indicating that this vaccine is of value in prevention of life-threatening *Pseudomonas* septicemia in severely burned patients when used in a course of extensive post-burn immunization. The potential value of this vaccine for prevention of life-threatening septicemias in patients undergoing anti-cancer or immunosuppressive therapy is also being explored in controlled clinical trials. Data from the present laboratory study of this vaccine will be utilized in formulating standards for control of this vaccine.

Proposed Course of the Project: If *Pseudomonas* vaccine becomes a licensed product, further studies on the vaccine described above will become largely a routine control function. Consideration is being given to the initiation of a program to investigate the possibility of producing nontoxic (or less toxic) immunogenic fractions from *P. aeruginosa*.

Honors and Awards: None

Publications: None

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Serial No. DBS 207
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Bioassay of Endotoxin in Bacterial Vaccines

Previous Serial Number: None

Principal Investigator: Kwei-Hay Wong, Ph. D.

Other Investigators: John C. Feeley, Ph. D.

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To establish a simple, sensitive, and reliable method for quantitation of endotoxin content in bacterial vaccines.

Methods Employed: Actinomycin D was used to potentiate endotoxicity in mice. Several strains of mice at different stages of growth were used. Results were compared with those from the mouse weight gain test for endotoxin content.

Major Findings: (1) Actinomycin D increased sensitivity of mice to the lethal effects of endotoxin by 500 to 2,000 times. NIH female mice weighing 20-25 grams were found most suitable. (2) Results obtained by titration with Actinomycin D were comparable to those from the mouse weight gain test. (3) Action of Actinomycin D seemed specific for endotoxin. (4) Other cancer chemotherapeutic drugs including cyclophosphamide, Methotrexate, 6 mercaptopurine, 5 fluoruracil, and mitomycin C did not have this potentiation capacity for endotoxin.

Significance for Bio-Medical Research and the Program of the Division: This method is useful for quantitation of sub-microgram amounts of endotoxin in either whole cell bacterial vaccines or extracts. It is extremely sensitive and, unlike the mouse weight gain test, is not affected by conditions of husbandry after the endotoxin is injected. LD_{50} can be easily calculated.

Proposed Course of Project: No further study is anticipated. Results will be published.

Honors and Awards: None

Publications: None

Serial No. DBS 101
1. Bacterial Products
2. Mycobacterial and Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Tuberculosis Vaccine

Previous Serial Number: Same

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigators: Dr. Thomas Wera (OD)
Sally R. Hedrick

Cooperating Units: Office of the Director

Man Years

Total:	0
Professional:	0
Other:	0

Project Description: No further work has been done on this project.

Proposed Course of Project: Analyze the results. Consider tests to assess immunogenicity of the vaccines. Determine stability of the vaccines to freeze-drying and various conditions of storage. Further characterization of the organisms for properties desirable in the selection of a reference or standard preparation.

Honors and Awards: None

Publications:

Chaparas, S. D., Wera, T. and Hedrick, S. R.: Antigens in culture filtrates of 12 BCG strains compared by electrophoretic, gel diffusion and skin tests. Proceedings of the BCG Symposium Frankfort, Dec. 1970. Permanent Section for Microbiological Standardization International Association of Microbiological Societies. (In press).

Serial No. DBS 103
1. Bacterial Products
2. Mycobacterial and Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hypersensitivity and Specificity of Fungal Diagnostic Substances for Dermal Tests

Previous Serial Number: Same

Principal Investigators:
Sotiros D. Chaparas, Ph. D.
Edward B. Seligmann Jr., Ph. D. (LCA)
Vernon Fuller, Ph. D. (LCA)

Cooperating Unit: Laboratory of Control Activities

Man Years

Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: (1) To develop suitable laboratory procedures for use in potency and identity tests for Fungal Diagnostic Substances for Dermal Tests. (2) To prepare standards for commonly used skin test reagents.

Methods Employed: Coccidioidin, Histoplasmin and Blastomycin were investigated for specific and heterologous skin reactivity using guinea pigs sensitized with the antigens alone or in combination with spores or hyphae, emulsified in complete or incomplete Freund's adjuvant. Coccidioidins prepared from spherules of *Coccidioides immitis* and by autolyses of mycelial mats in synthetic medium will be investigated for specificity and suitability as standards. Antigenic makeup and skin reactivity will be examined.

Major Findings: No new findings. Results have been confirmed and statistically analyzed.

Significance to Bio-Medical Research and the Program of the Division: (1) Specific assays would be helpful in the development and improvement of standards for Fungal Diagnostic Substances for Dermal Tests. (2) Suitable standard preparations permit the Division to better control commercial preparations for uniformity and consistency of production.

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Proposed Course of Project: (1) Contract for manufacture of Coccidioidin under various conditions of growth has been let. These preparations will be examined for antigenic content, specificity, skin reactivity and chemical content. Their suitability as standards for Coccidioidin will be evaluated.

Honors and Awards: None

Publications: None

Serial No. DBS 135
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mycobacterial Antigens: Immunological Specificity and Antigenic Analysis

Previous Serial Number: Same

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigators:
Sally R. Hedrick
Harold Baer, Ph. D.
Henry Godfrey, M. D.
Edward B. Seligmann Jr., Ph. D. (LCA)
Clifford J. Maloney, Ph. D. (OD)
Daniel E. Thor, M. D., Ph. D. (LVR)

Cooperating Units:
Office of the Director
Laboratory of Control Activities

Man Years

Total:	1.5
Professional:	0.7
Other:	0.8

Project Description: This project is divided into III parts.

Objectives: The overall objectives are: (1) to determine the specificity, potency and identity of various mycobacterial antigens; (2) to determine reactivity of licensed tuberculins in guinea pigs sensitized with "atypical" mycobacteria; (3) to classify various mycobacteria; (4) to compare the *in vivo* and *in vitro* reactivity of fractions prepared from BCG culture filtrates.

Part I. *In vivo* studies of the specificity of antigens prepared from various mycobacterial species. (In collaboration with Dr. Maloney.)

Methods Employed: Antigens from mycobacterial species have been studied by delayed skin reactivity, lymphocyte transformation, acrylamide gel electrophoresis, immunoelectrophoresis, and the Ouchterlony double diffusion in gel reactions.

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Major Findings: (a) When a standard reference serum against M. tuberculosis was used to study antigens of homologous and heterologous species of Mycobacterium by immunoelectrophoresis, it was possible to identify eleven precipitin arcs. The most cathodic seven antigens were found to be shared amongst most species tested. (b) Mycobacteria of the avium-intracellulare complex could readily be differentiated on the basis of reactivity in homologously and heterologously sensitized animals. Differentiation of these two species by other means is very difficult.

Part II. Reactivity of U.S. licensed tuberculins in guinea pigs sensitized with "atypical" mycobacteria. (In collaboration with Dr. Seligmann.)

Methods Employed: A number of mycobacterial species have been used as sensitizing agents to establish profiles of reactivity in guinea pigs for the Standard OT and PPD tuberculin preparations and tuberculins from manufacturers. The sensitizing organisms include BCG, M. intracellulare (Battey), M. kansasii, M. scrofulaceum (Gause) and M. fortuitum.

Major Findings: On the whole, the reactivity profiles of all the tuberculins tested were rather similar. One manufacturer's product seemed to react less than the other's in guinea pigs sensitized with M. kansasii and M. fortuitum.

Part III. In vivo and in vitro reactivity of fractions prepared from BCG culture filtrates (with Drs. Thor, Godfrey and Baer).

Methods Employed: The reactivity of various nondialyzable and dialyzable substances were studied by skin test, inhibition of macrophage migration and by lymphocyte transformation.

Major Findings: Nondialyzable fractions rich in carbohydrates or rich in proteins were equal in skin reactivity and macrophage inhibition activity. The degree of lymphocyte transformation seemed to be solely dependent on protein content. The activity of the dialyzable fractions in the 3 tests seemed to be correlated with molecular size.

Significance to Bio-Medical Research and the Program of the Division: These studies underscore the complex nature of mycobacterial antigens from different species and account for some of the difficulties experienced in attempts to prepare specific skin reactive substances. Delayed skin reactivity appears to be a very powerful tool for differentiating various mycobacteria and should be useful in clarifying their taxonomic status. The apparent unreactivity of carbohydrate antigens in the lymphocyte transformation test emphasizes caution in the use of this test for skin reactive substances which contain significant concentration of carbohydrate. The above studies are related to the improvement of standards and procedures for evaluating Diagnostic Substances for Dermal Tests.

Proposed Course of Project: Further studies of the relationships of mycobacteria by skin test and immunoelectrophoresis are planned. Studies of differences between tuberculin preparations of different manufacturers will be extended. Elaborate on specificity differences between mycobacterial antigens, especially between Battey and tuberculosis organisms.

Honors and Awards: None

Publications:

Chaparas, S. D., Hedrick, S., Garman, R., and Clark, R.: Comparison of the lymphocyte transformation test with the tuberculin test in rhesus monkeys and chimpanzees. Amer. J. Vet. Research 31:1437-1441, 1970. (Previously reported as "In Press.")

Chaparas, S. D., Thor, D. E., Godfrey, H. P., Baer, H. and Hedrick, S. R.: Tuberculin-active carbohydrate that induces inhibition of macrophage migration but not lymphocyte transformation. Science 170:637-639, 1970.

Chaparas, S. D. and Maloney, C. J.: Comparison of relationships of mycobacteria by skin tests and *in vitro* lymphocyte transformation. Mycobacterial Taxonomy Symposium - to be published in Proceedings of the X International Congress for Microbiology.

Chaparas, S. D., Thor, D. C. and Hedrick, S. R.: Comparison of lymphocyte transformation, inhibition of macrophage migration and skin tests using dialyzable and nondialyzable tuberculin fractions from *Mycobacterium bovis* (BCG). J. Immunol. (Accepted for publication)

Serial No. DBS 160
1. Bacterial Products
2. Mycobacterial and
Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Delayed Skin Reactivity and Lymphocyte Transformation in Monkeys with Experimental Allergic Encephalitis (EAE)

Previous Serial Number: Same

Principal Investigators: Sotiros D. Chaparas, Ph. D.
Ronald G. Clark, Ph. D. (LP)

Other Investigator: Sally R. Hedrick

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description: This project has been terminated.

Honors and Awards: None

Publications:

Chaparas, S. D., Clark, R. G., Kies, M. W. and Hedrick, S.: Transformation of lymphocytes from monkeys with Experimental Allergic Encephalomyelitis. Res. Communic. in Chem. Pathol. and Pharmacol. 1:637-652, 1970.

Serial No. DBS 17
1. Bacterial Products
2. Mycoplasma
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mycoplasma in Human Disease and Biologics

Previous Serial Number: Same

Principal Investigator: Michael F. Barile, Ph. D.

Other Investigators:
Ruth L. Kirschstein, M.D. (LP)
Stanley H. Singer, M.D. (LP)
Joseph G. Tully, Ph. D. (NIAID)
Richard A. DelGiudice (HRC)
Theodore R. Carski, M.D. (HRC)
Donald B. Riggs
Marion W. Grabowski

Cooperating Units:
Laboratory of Pathology, DBS
Laboratory of Microbiology, NIAID
Huntingdon Research Center, Inc., Baltimore

Man Years

Total:	2.7
Professional:	0.7
Other:	2.0

Project Description:

Objectives: (1) To examine the biology, metabolism, and pathogenicity of Mycoplasma. (2) To develop new methods or improve existing methods for primary isolation and detection of Mycoplasma as they pertain to the test for Mycoplasma required for certain biologic products. (3) To investigate the role of Mycoplasma in diseases of man and animals.

Methods Employed: (1) Several different broth, semi-solid agar-broth and agar media containing various supplements were employed and cultures were incubated aerobically and in a carbon dioxide-nitrogen atmosphere. (2) Mycoplasma species were identified on the basis of their serologic, metabolic, pathogenic and membrane protein acrylamide electrophoretic mobility properties. (3) Several cell cultures were infected with either mycoplasmas and/or viruses and determinations were made on the effect of mycoplasma on interferon production and on virus yields.

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Major Findings: 1. Continuing studies on mycoplasma-virus-cell culture interactions using primary and continuous cell cultures derived from mouse tissues indicate that a) mycoplasmas can interfere with interferon activity resulting in higher virus yields and this activity is not cell dependent; b) arginine-utilizing mycoplasma reduce yields of arginine-requiring viruses and c) mycoplasmas can produce toxic cytopathic effects in cell cultures resulting in reduced virus yields.

2. A new simian Mycoplasma species, designated M. primatum, was characterized on the basis of its biological, biochemical and serologic properties and cell membrane protein electrophoretic patterns. The 18 strains isolated from oral and urogenital tissue of the African green monkey, Cercopithecus aethiops, and one strain isolated from man were related to each other and unrelated to the 40 well-established species of Mycoplasma and Acholeplasma.

3. A new canine Mycoplasma species, designated M. edwardii, was characterized. The 59 strains studied were isolated from the oral and genital tissues of pure-bred beagles and of mongrel street dogs.

4. Mycoplasmas were isolated from 73 of 379 (19%) commercial bovine serum lots examined. Of 31 strains examined for identity, 28 were identified as M. arginini and 3 strains were unclassified and may represent a new species. Data indicate that M. arginini, a bovine strain, is a common contaminant of commercial bovine sera and of cell cultures, and that bovine serum is a major source of bovine mycoplasma contamination of cell cultures.

Significance to Bio-Medical Research and the Program of the Division: These studies may provide information on the pathogenicity of mycoplasmas for man and animals. The improved culture procedures developed are applicable to the isolation and detection of mycoplasmas in biologics which are subject to mycoplasma-contamination risk.

Proposed Course of Project: To pursue studies to improve methods for primary isolation of Mycoplasma from biologics and from tissues associated with disease. To consider assay methods for requirements for Mycoplasma vaccine products.

Honors and Awards: None

Publications:

Barile, M. F., DelGiudice, R. A., Carski, T. R., Yamashiroya, H. M. and Verna, J. A.: Isolation and rapid identification of Mycoplasma species from canine tissues by plate immunofluorescence. Proc. Soc. Exp. Biol. Med. 134:146-148, 1970.

Serial No. DBS 104
1. Bacterial Products
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Pertussis Vaccine

Previous Serial Number: Same

Principal Investigators: Charles R. Manclark, Ph. D.
Margaret Pittman, Ph. D.

Other Investigators: James F. Marshall

Cooperating Units: Pharmaceutical Manufacturers' Association

Man Years

Total:	2.9
Professional:	1.1
Other:	1.8

Project Description:

Objectives: The overall objective is that the pertussis vaccine component of products issued by U.S. licensed establishments has optimum properties: adequate and stable potency through the dating period and relative freedom from untoward reactivity. Objectives of current studies are (1) to relate laboratory assayed reactivity of vaccines and fractions of the constituent bacteria, as determined by multiple tests, to clinical reactivity; (2) to find the cause of the neurological reactions that on rare occasions follow pertussis vaccination, and to develop a laboratory assay that measures this reactive factor, should it be identified.

Methods Employed: Mouse potency tests to determine adequacy and stability of potency. Analysis of results at regular intervals to determine any reflected trend. Measurement of pertussis vaccine toxicity (reactogenicity) by determination of the early and late weight changes in mice, the lethal dose in mice, histamine sensitizing activity, necrotizing activity in the skin of suckling mice, capacity to induce edema in foot pad of the rat, rabbit pyrogenicity, ability to promote lymphocytosis and/or leucocytosis in the mouse, etc.

Physical and chemical methods for isolation and concentration of the lymphocyte promoting factor (LPF) from Bordetella pertussis (1) culture supernatants including ultrafiltration, freeze-drying, and carboxymethyl cellulose ion exchange chromatography and (2) cells broken in a Braun homogenizer and extraction by physical-chemical procedures.

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Major Findings: Subproject 1: No significant changes in potency and toxicity of pertussis vaccine submitted for release were noted.

LPF was effectively concentrated but its isolation from the supernatants was not satisfactory. Results using broken cells are as yet incomplete. A comparative study of the LPF content of vaccines produced by licensed manufacturers has been completed.

Subproject 2: The collaborative DBS-PMA laboratory assays of the two study vaccines have been completed. The results provide information on the reproducibility of tests within and between laboratories which could be analyzed statistically. The results, also, point up the need for a revision in the prescribed mouse toxicity test in Regulations. The quality of mice now available to manufacturers have so improved since the 3-gram weight gain was prescribed that this specification is not as discriminatory as formerly. The findings of the cooperative study and analyses of routine tests of lots of vaccine suggest that the toxicity test include the use of a toxicity reference vaccine and that the acceptable weight gain be expressed as a prescribed percentage of the weight gain of the saline-control mice.

Significance to Bio-Medical Research and the Program of the Division: The projects are related directly to the control of the potency and safety of pertussis vaccine and the relation of laboratory assayed toxicity to untoward reactivity in children. Encephalopathy that follows vaccination, in rare instances, is a distressing reaction and the experimental studies are directed toward the elucidation of the cause. These projects also provide basic immunological information on Bordetella pertussis.

Proposed Course of Project: (1) To continue the testing and surveillance of potency and safety of pertussis vaccine. (2) To prepare for publication the results of the cooperative DBS-PMA study. (3) To analyze the results of the measurement of the LPF content of pertussis vaccines with the view to determine their applicability to the safety of pertussis vaccine and suitability for publication. (4) Take preparatory steps toward revision of the Mouse Toxicity Test. (5) To isolate and characterize the protective antigen of Bordetella pertussis as a necessary first step towards the improvement of pertussis vaccine. (6) To initiate studies to determine the actual incidence and nature of clinical reactions to the administration of pertussis vaccine. (7) To initiate research to determine physiologic factors involved in the growth of Bordetella pertussis that affect the potency and toxicity of pertussis vaccine.

Honors and Awards: None

Publications:

Pittman, M.: Pertussis Vaccine Testing. Proceedings of the Round Table Conference on Pertussis, Bilthoven, Holland, 1969. Symposium Series Immunobiol. Stand. Basel/New York, Karger, 1970, vol. 13, pp. 65-68.

Serial No. DBS 138
1. Bacterial Products
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Selective Breeding to Establish a "Standard" Mouse

Previous Serial Number: Same

Principal Investigators: Margaret Pittman, Ph. D.
Carl T. Hansen, Ph. D. (DRS)
Charles R. Manclark, Ph. D.

Other Investigators: James F. Marshall
William J. Hinkle (DRS)

Cooperating Unit: Division of Research Services

Man Years

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: To establish a mouse strain that has superior and consistent characters for the potency and toxicity assays of biologics, particularly bacterial vaccines.

Methods Employed: Thirty-seven breeding pairs of a "sensitive" line and twenty-two pairs of a "resistant" line have been selected and bred. The progeny of each are being tested for their ability to be sensitized to histamine by pertussis vaccine. Mice with the highest ability as well as those with the highest resistance are being selected for further breeding.

Major Findings: (1) The colony is now in its tenth generation with a few of the breeding pairs in the seventh, eighth, or ninth generation. (2) The evidence is clearcut that both sensitivity and resistance to histamine sensitization by pertussis vaccine is genetically determined. (3) Selectively bred animals of the sensitive line now manifest a 70% sensitivity compared to the 31% of the starting colony. Mice of the resistant colony demonstrate only a 3-4% sensitivity. (4) The variability of sensitivity has decreased from a standard deviation of ±20% in the original colony to ±15% in the present sensitive line.

Significance to Bio-Medical Research and the Program of the Division: The availability of a "standard" mouse for potency and toxicity assays of pertussis vaccine and other bacterial products should decrease variability in tests and give greater assurance of the validity of the estimate of potency and toxicity of the products.

Proposed Course of Project: (1) To continue the study as described above. (2) To study the nature and inheritance of the genetic markers which determine the ability of a mouse to be sensitized to histamine. (3) To pursue basic immunogenic studies directed toward the establishment of an isogenic strain of mouse which carries the genetic marker(s) for histamine sensitivity and reactivity to pertussis protective antigen(s). (4) To determine if the difference is quantitative, mice will be selectively bred to raise or lower the threshold of activity in HSF-S and HSF-R mice, respectively. (5) To study the feasibility of using F_1 hybrids of two different HSF-S strains to retain the "sensitive" character of inbred strains and yet obtain the advantages of hybrid vigor resulting from such a first generation cross. (6) The data collected to this point will be prepared for analysis by automatic data processing methods. The initial breeding program and data analysis should be completed by October 1971.

Honors and Awards: None

Publications: None

[illegible]
LBBP
LCA
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LVR
LVI
Adjuncts to OD
Contracts

Annual Report of the
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1970 through June 30, 1971

The continuing impetus of new products, new establishments and new improved techniques in the blood banking world has had its impact on the functions of the Laboratory of Blood and Blood Products. Fortunately, Dr. Ashworth, after an absence of five years agreed to return in March as Chief of the Laboratory, so for the first time in four years, there is now full time leadership, releasing Dr. Gibson to spend his entire time as Assistant Director. Unfortunately, Dr. Esposito has resigned as Acting Chief of the Diagnostic Reagents Section, a position he has filled with accomplishment and distinction.

I. Intramural Research Activities: The research program of the Laboratory included areas directly related to the control responsibilities of each of the individual sections—Blood and Blood Derivatives, Coagulation, and Diagnostic Reagents.

The former has been concerned primarily with albumin and immunoglobulin, especially from the standpoint of purity and stability. Particular emphasis has been placed on development of methods for predicting the stability of such products and for assessing the extent of deterioration during storage. Research on coagulation has dealt with Factors I, VII, VIII, IX, X, XI_a, and XIII, as well as with activators affecting these Factors, in an attempt to bring about an understanding of their chemistry which can be used in the design of improved potency assays. Investigations dealing with diagnostic reagents have been directed toward developing a simple and reliable radioimmunoassay for anti-Rh_o (D), assembling a panel of well characterized histocompatibility antigens for potential use in determining the specificities of antisera and establishing the validity of serological and biological assays for the potency of antilymphocytic serum.

II. Other Than Research Activities: During the past year, the development of a diagnostic reagent to screen donors for the presence of Hepatitis Associated (Australia) Antigen has provided blood banks with a positive means of attacking the ever present danger of transfusion transmitted hepatitis. Through the combined efforts of LVI and LBBP, Additional Standards for Hepatitis Associated Antibody (Anti-Australia Antigen) were established and licenses have now been issued for this product. Although the sensitivity of existing procedures eliminates only approximately 25% of the potentially infective donors, this reagent represents an important milestone in increasing transfusion safety.

The first license for Cryoprecipitated Antihemophilic Factor (Human) was granted in September 1970 and the ensuing large number of requests is some indication of the widescale use of this effective agent which is relatively simple to produce. 241 lots of this product alone have been tested as prelicensing samples.

The first Red Blood Cells (Human) Frozen and Red Blood Cells (Human) Deglycerolized prepared under license were distributed for use, making it possible to extend the dating period from 21 days to two years, an event long awaited by personnel harrassed by donor recruitment for emergencies.

The licensing of these new products contributed to the unprecedented number of applications and amendments processed in the Laboratory. Of the 426 licensing actions received in the Division, 365 were assigned to the Laboratory of Blood and Blood Products to review and propose recommendations. This activity has necessarily consumed a major proportion of the staff's time and efforts. See Table I.

The number of lots submitted for control testing also reflected this increased activity. The control tests performed on blood products by this Laboratory exceeded 29,000, with some 5,000 lots having been received. Diagnostic antiserums represented a major percentage of this total, for every filling of these licensed serums is subjected to a variety of tests prior to its distribution by the manufacturer. See Table III.

There are ten authorized inspectors and three inspector designees currently on the Laboratory of Blood and Blood Products staff. This group visited 393 locations, which required each of the senior inspectors to be on travel orders an average of twenty-eight working days. See Table II. 27 biological manufacturers not previously holding an establishment license applied, and a number of licensed establishments moved to new locations. Since these actions involved inspection of the facilities as they became completed and operational, a number of unscheduled visits were made in addition to the assigned scheduled trips. In all cases the manufacturer was accommodated by meeting his date of anticipated readiness.

The Laboratory has continued to push ahead in its goal of completing Additional Standards for all of its products. During the year, Standards for Hepatitis Associated Antibody (Anti-Australia Antigen), Cryoprecipitated Antihemophilic Factor (Human), Whole Blood (Human) Modified with Cryoprecipitated Antihemophilic Factor (Human) Removed were added to the Regulations and Standards for Platelet Concentrate (Human) were published as a Notice of Rule Making. New drafts of Tentative Standards for the following were sent to manufacturers and other interested parties for comments:

Rh Typing Serum
Reagent Human Erythrocytes
Anti-Human Serum
Blood Grouping Serum
Thrombin

Additional Standards for Normal Serum Albumin and Plasma Protein Fraction have been forwarded for publication as a Notice of Proposed Rule Making. It is hoped that by the end of this next fiscal year all of these will have been incorporated into the Regulations.

Despite the pressures imposed because of personnel ceilings, the staff of the Laboratory has been encouraged to take advantage of the excellent training opportunities available to them, both on and off the National Institutes of Health reservation. Tuition assistance has been granted to three of the staff who are attending local universities taking job-related courses. The Division has provided funds and the Laboratory has granted time for eight individuals to participate in fifteen courses, most of these offered by Graduate Program, or by the Office of Training and Employee Development and covering a variety of subjects from Scientific Etymology to Transplantation and Immunology, and from Introductory Course for Supervisors to Advanced Course for Supervisors. This amount of personnel training was achievable not only because of the interest and application of the participants who requested permission to take the courses, but because of the support and cooperation afforded them by their supervisors and fellow workers in making it possible for them to attend.

Lastly, the sessions planned for the total staff and the inspector group were continued. Under Dr. Finlayson's leadership, staff members periodically reported on the status of technical and research projects, and the discussions that ensued provided critiques and suggestions for future pursuits. At the regularly held inspector meetings, presided over by the Chief, the group reviewed experiences and attempted to achieve a consistent informed approach to their blood bank inspections. Other staff from the Division have been invited to attend these meetings when subjects of mutual concern were presented. These subjects have included Diagnostic Reagent Manufacturing Practices, Hepatitis Associated Antigen Testing, and the Use of Automated Equipment in Blood Banks.

This has been a productive, stimulating and exceptionally busy year.

STATUS OF LICENSE APPLICATIONS

April 1, 1970 through March 31, 1971

TABLE 1

	ESTABLISHMENT APPLICATIONS	PRODUCT APPLICATIONS	AMENDMENTS	TOTAL
No. Pending 4-70	12	41	93	146
No. Received	27	137	201	365
No. Recommended	8	51	179	238
No. Withdrawn or Inactive	6	14	21	41
No. Pending 3-71	25	113	94	232

CONTROL TESTING OF BLOOD AND BLOOD PRODUCTS

April 1, 1970 through March 31, 1971

TABLE II

	LOTS TESTED			Total No. Lots	TESTS PERFORMED			Total No. Lots
	Pre-licensing	Inspection	For Release		Purity	Potency	Safety	
Blood Fractions	218	85	1858	2161	1109	809	2250	4168
Diagnostic Reagents	329	162	1499	1990	10208	6695	0	16903
Whole Blood, Single Donor Plasma, Red Blood Cells, Cryoprecipitated AHF	270	585	0	855	0	0	8084	8084
TOTALS	817	832	3357	5006	11317	7504	10334	29155

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Contracts

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CONTROL ACTIVITIES REQUIRING TRAVEL STATUS

April 1, 1970 through March 31, 1971

TABLE III

ACTIVITY	LOCATIONS	CONTRACTS	MAN DAYS OF TRAVEL
Inspections	393		311
Support of Contracts		2	4

Serial No. (NIH-DBS) 33
1. Blood & Blood Products
2. Blood & Blood Derivatives
   Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Preparation, Characterization, Stability and Potency of Blood Products

Previous Serial Number: Same

Principal Investigators:
Pinya Cohen, Ph.D.
John S. Finlayson, Ph.D.
Andrew M. Young, M.S.

Other Investigators:
B. Lynne Armstrong
David L. Aronson, M.D.
Herman Hudson
Jeanne Lacerte, A.B.
Mae Moody
Joan Roelands, B.A.

Man Years:
Total: 3.1
Professional: 2.4
Other: 0.7

Patient Days: None

Project Description:

Objectives: To obtain more detailed information on the characteristics of blood products, to determine changes in their properties which occur during processing and storage, and to develop improved methods and standards.

Methods Employed: Standard serological, immunological and physicochemical methods have been used. Additional specialized methods which are under development are described in the following section.

Major Findings: a) Comparative analyses of Normal Serum Albumin (Human) done by disc and Tiselius moving boundary electrophoresis have shown a high degree of correlation. Disc electrophoresis is reproducible, fast and accommodates many more samples than Tiselius electrophoresis. Disc electrophoresis is therefore being used to test samples of albumin lots sent to this Division for release. (Moody and Cohen)

b) Immunologic techniques have been used to analyze placental Immune Serum Globulin (Human) and placental Normal Serum Albumin (Human) for extraneous protein. Experiments with rabbit antiserum produced against a purified placental protein showed that globulin and albumin are contaminated with a placental component. However, using different rabbit antisera against the same purified antigen, globulins and albumin appear to be contaminated with different components or a common component with more than one antigenic determinant. The placental component associated with globulin was found in Immune Serum Globulin (Human) from one manufacturer but not in the Immune Serum Globulin (Human) of two others. All placental albumins contained a common component as revealed by antiserum against the purified placental protein. (Roelands and Cohen)

c) Immune globulins prepared from placentas, venous plasma and mixtures of the two sources were assayed by hemagglutination inhibition for blood group substances A and B. The maximum amount of B substance in placental Immune Serum Globulin (Human) was 200 μg/ml; in venous Immune Serum Globulin (Human), 5 μg/ml; and in Immune Serum Globulin (Human) from mixed sources, 27 μg/ml. In placental globulins the amount of blood group substance A was approximately 10% of the amount of B substance. Tetanus Immune Globulin (Human) from venous plasma contained levels of blood group substance similar to those in Immune Serum Globulin (Human) from mixed sources. (Cohen and Hudson)

d) Tests have been conducted on Immune Serum Globulin (Human), cryoprecipitates, and Antihemophilic Factor (Human) to determine the presence of isoagglutinins which in high titer could cause clinical reactions. The immune globulins and cryoprecipitates contained low levels of both saline-agglutinating antibodies and those detectable by the indirect Coombs' test, but the Antihemophilic Factor (Human) from one manufacturer contained significantly higher levels of the latter. As a result of tests by the manufacturer and DBS, the manufacturer has set upper limits for the amount of isoagglutinins in Antihemophilic Factor (Human). (Lacerte and Cohen)

e) Ultracentrifugation of a sampling of 31 in-date lots of Immune Globulin (Human) products revealed that 9 contained 3.5 S material, with 6 of these containing more than 10% 3.5 S material. (Young)

f) A prospective study of the breakdown of Immune Globulin (Human) products during storage was undertaken in an attempt to devise a test by which the stability of such products could be predicted. The experimental design was based on the assumption that plasmin is the primary enzyme responsible for proteolytic degradation. Aliquots of each sample were placed in storage at -23° C, 5° C, room temperature, and at room temperature in the presence of urokinase. Preliminary results obtained by examination of the latter indicated a correlation between the amount of plasmin detectable by the fibrin plate method and the appearance of 3.5 S material after 30 days at room temperature. Aliquots stored without urokinase will be examined by ultracentrifugation after 1 year of storage. (Young and Aronson)

g) Application of a variety of analytical methods to a series of Immune Serum Globulin (Human) preparations which had undergone 1) no degradation, 2) spontaneous degradation, 3) plasmin-induced degradation or 4) urokinase-induced degradation indicated the following order of sensitivities: immunoelectrophoresis (most sensitive) > acrylamide gel electrophoresis in sodium dodecylsulfate > ultracentrifugation >> isoelectric focusing in polyacrylamide gel slabs. Exclusion chromatography on Sephadex G-200 is very sensitive and can be quantitated, but it will accommodate only one sample at a time. Acrylamide gel electrophoresis in sodium dodecylsulfate has the advantage over disc electrophoresis (see section a) of this Report) that migration is independent of electrophoretic mobility and therefore clearly reflects molecular size changes. Immunoelectrophoresis, though less amenable to quantitation than the other techniques, offers sufficient sensitivity and convenience to serve as a routine screening procedure. (Finlayson, Young and Armstrong)

h) The effect of mild heating (45° C for 72 hours or less) on the stability of Immune Serum Globulin (Human) during subsequent storage at 5° C was determined in a small number of samples fractionated from venous plasma. Results confirmed the findings of a much larger study involving "accelerated storage" at 24° C (see Annual Report, 1969). In some cases the effect was dramatic, the extent of degradation being reduced from approximately 50% in the unheated samples to 1% or less in the heated ones. (Finlayson and Young)

i) Since mild heating was found to inhibit proteolytic degradation during subsequent storage of Immune Serum Globulin (Human), a study was undertaken to determine whether heating of whole serum prior to storage exerts a similar inhibitory effect. The first phase of this study, involving single-donor serum, has been completed. Unheated controls and samples heated under a variety of conditions (varying from 45° C for 36 hours to 50° C for 72 hours) were stored at temperatures ranging from -23° C to 37° C. The rapidity of onset and the extent of degradation of IgG (as assessed by immunoelectrophoresis) was a direct function of temperature. No effect of prior heating on stability was detected except possibly in samples stored at 5° C, where heating may have slightly accelerated degradation. (Finlayson and Armstrong)

j) A study utilizing exclusion chromatography and ultracentrifugation demonstrated that heating commercial Immune Serum Globulin (Human) at 37° C or 45° C brought about a decrease in the amount of immunoglobulin G dimer. This decrease could occur in the absence of fragmentation and aggregation. It was shown to reflect a reversible, temperature-dependent monomer-dimer transition which apparently involved all or most of the immunoglobulin G present. Individual preparations of immunoglobulin G were found to vary greatly in propensity to dimerize. (Finlayson, Armstrong and Young)

k) A tabulation of the results obtained during the examination of blood products prepared by licensed blood banks over the past 8 years has been initiated. The purpose of this study is to determine which reasons for failure to comply with the Regulations occurred most frequently. Once the nature and frequency of these deficiencies has been established both licensed and unlicensed blood banks will be advised of the recurring problem areas where improvements should be made. (Lacerte and Cohen)

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. Such surveillance involves the possession of knowledge regarding the stability of biological products as well as the availability of appropriate control test methods and technical standards. The project fulfills these needs by providing information leading to more realistic dating periods and control test methods.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Serial No. (NIH-DBS) 81
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Clotting and Fibrinolytic Systems

Previous Serial Number: Same

Principal Investigator: David L. Aronson, M.D.

Other Investigators: B. Lynne Armstrong
John S. Finlayson, Ph.D.
Anne J. Mustafa

Cooperating Units: National Heart and Lung Institute (Dr. John J. Pisano)
Clinical Center (Dr. Harvey R. Gralnick)
National Institute of Dental Research (Dr. Soo I. Chung)

Man Years:
Total: 3.0
Professional: 1.3
Other: 1.7

Patient Days: None

Project Description:

Objectives: To purify and characterize components of these systems and to develop an understanding of their chemistry.

Methods Employed: The methods employed ranged from measurements of clotting factors *in vitro* (by standard assay systems and by those developed in this Laboratory) to fractionation and analytical procedures typical of protein biophysics and biochemistry.

Major Findings: a) Further validation and characterization of the Factor IX assay (see Annual Report, 1970) has been achieved. Other laboratories are now using this method and find it satisfactory. Since reports from a preliminary study involving the analysis of a potential Factor IX reference preparation by several laboratories indicated great variability, a cooperative study has been undertaken. In conjunction with the International Committee on Haemostasis and Thrombosis, samples containing Factor IX have been prepared and sent to many laboratories for testing. (Aronson)

b) It was found that Factors VII and IX could be removed from Factor X preparations by treating with chloroform. (Experiments have been undertaken to determine whether this removal occurs by destruction of Factors VII and IX or through a bona fide separation.) Factor X treated in this manner is converted to active Factor X (X_a) more slowly than are conventional Factor X preparations, regardless of the activating agent—intrinsic system, extrinsic system or sodium citrate. The so-called "citrate activation" apparently involves Factor VII, inasmuch as chromatographic removal of residual Factor VII from $CHCl_3$-treated Factor X results in a Factor X preparation which in the presence of 25% sodium citrate is converted to X_a at an extremely low rate. (Aronson and Mustafa)

c) Continued studies on the two chromatographically separable types of human fibrinogen have shown no differences between these types in the molecular size of the individual polypeptide chains. Tryptic peptide mapping has confirmed the initial finding that there were no marked qualitative differences in the maps of the α(A)- and β(B)-chains. However, the γ-chain isolated from rechromatographed peak 2 fibrinogen exhibits electrophoretic heterogeneity. Tryptic peptide mapping of polypeptide chains isolated by methods other than sulfitolysis is indicated. (Finlayson and Armstrong)

d) A congenitally abnormal fibrinogen discovered by Dr. Gralnick was characterized with respect to clotting behavior, metabolism, and immunological, ultracentrifugal, electrophoretic, chromatographic, and biochemical properties. Compared with normal fibrinogen, this fibrinogen (fibrinogen Bethesda) showed a pronounced decrease in the rate of fibrinopeptide release by thrombin, a functional defect which served to differentiate it from all other dysfibrinogenemias reported. This decrease was shown to involve both fibrinopeptides A and B. (Finlayson, Gralnick and Armstrong)

e) By means of the enzymic method for direct determination of the dipeptide which forms the covalent crosslinks (see Annual Report 1969) it was demonstrated that fibrin clots formed in plasma from individuals with Factor XIII deficiency contained little or none of the ε-(γ-glutamyl)lysine crosslink whereas those formed in normal plasma contained approximately six moles of the crosslink per mole of fibrin. Partial supplementation of the deficient plasma with Factor XIII commensurately increased the number of crosslinks. (Finlayson, Pisano and Armstrong)

f) Chromatography of guinea pig fibrinogen on DEAE cellulose revealed the presence of two major fractions. This similarity to human fibrinogen was utilized in a series of comparisons between Factor XIII and guinea pig tissue transglutaminase, which (like active Factor XIII) can catalyze the formation of crosslinks in fibrin. Guinea pig Factor XIII, like human Factor XIII, is co-eluted with peak 2 fibrinogen, thus permitting the isolation of a Factor XIII-free fibrinogen subfraction. Tissue transglutaminase (which does not occur in the blood naturally) binds readily to fibrinogen, both in vivo and in vitro. Experiments in vitro have shown that it will bind to either peak 1 or peak 2 fibrinogen. On the other hand, no evidence of such binding between Factor XIII and fibrinogen has been obtained. (Finlayson, Chung and Armstrong)

g) By the use of the [^{14}C]putrescine incorporation test (Dvilansky _et al_., _Brit_. _J_. _Haematol_. 18:399, 1970), purified human thrombin, Factor X_a, Factor XIII, and specific enzyme inhibitors, it was established that thrombin activates Factor XIII whereas Factor X_a does not. Thus the action ascribed to thrombin by many investigators is due to thrombin _per se_ rather than to contamination with Factor X_a. The pattern and extent of fibrin crosslinking brought about by clotting Factor XIII-rich fibrinogen with thrombin or a coagulant fraction from the venom of _Bothrops atrox_ (_cf_. project 82) was compared by polyacrylamide gel electrophoresis in the presence of dissociating agents. No differences were noted, indicating that despite the differences between the specificities of these enzymes in catalyzing fibrinopeptide release, their actions in Factor XIII activation are similar. (Aronson and Chung; Finlayson and Pisano)

Significance to Bio-Medical Research and the Program of the Division: Control of the purity and potency of therapeutic blood derivatives used to treat coagulation and thrombotic disorders involves detailed understanding of the mechanism of the clotting and fibrinolytic porcesses and the chemistry of the assay methods. This understanding is used in improving the validity and precision of control tests.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Pisano, J.J., Finlayson, J.S., and Peyton, M.P.: ε-(γ-Glutamyl)lysine, crosslink of polymerized fibrin. _Thromb_. _Diath_. _Haemorrh_. _Suppl_. 39:112-121, 1970.

Pisano, J.J., Finlayson, J.S., Peyton, M.P., and Nagai, Y.: ε-(γ-Glutamyl)lysine in fibrin: lack of crosslink in factor XIII deficiency. _Proc_. _Nat_. _Acad_. _Sci_. _USA_. 68:770-772, 1971.

Serial No. (NIH-DBS) 82
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Proteins in Body Fluids

Previous Serial Number: Same

Principal Investigator: David L. Aronson, M.D.

Other Investigator: Henry Webster (guest worker)

Cooperating Units: National Institute of Dental Research (Dr. Soo I. Chung)

Man Years:
Total: 0.4
Professional: 0.2
Other: 0.2

Patient Days: None

Project Description:

Objectives: To complement available data on blood proteins with a reservoir of basic information on the properties of and methods suitable for use on the proteins of blood and other fluids, including proteins involved in the clotting and fibrinolytic systems (see projects 33 and 81).

Methods Employed: The fractionation methods are those typically used in protein separations. Analytical methods include physical, chemical and spectrophotometric procedures as well as enzyme assays.

Major Findings: Chromatographic separation of the proteins in snake venoms yielded a number of different fractions, several of which exhibited enzymic activity. Attention was focused on fractions with proteolytic activity; these could be differentiated on the basis of substrate specificity. A preparation from the venom of Akistrodon contortrix contortrix (southern copperhead) was found to have thrombin-like activity. However, the clots which it produced with Factor XIII-rich human fibrinogen were soluble in 2% acetic acid and thus presumably contained little or no covalent crosslink. In contrast, a coagulant fraction prepared from the venom of Akistrodon rhodostoma (Malayan pit viper) was shown to activate Factor XIII and thus to be capable of producing clots which are insoluble in 2% acetic acid. Although this latter finding contradicts earlier published reports, it has now been confirmed in two other laboratories.

Significance to Bio-Medical Research and the Program of the Division: The control program of the Laboratory requires information regarding the characteristics of proteins and methods used in studying them. The basic research program described above continues to provide the staff with a constantly updated pool of fundamental information concerning the methodology and properties of proteins.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Serial No. (NIH-DBS) 176
1. Blood & Blood Products
2. Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunosuppression: Anti-Lymphocytic Serum and Rh_o (D) Immune Globulin

Previous Serial Number: Same

Principal Investigator: Vito M. Esposito, Ph.D.

Other Investigators: George W. Morgan, Ph.D.
Elizabeth B. Paul, A.B.
Mildred L. Woodward, B.S.

Man Years:
Total: 1.3
Professional: 1.3
Other: 0

Patient Days: None

Project Description:

Objectives: The overall objectives are to develop potency assays that reflect the efficacy of anti-lymphocytic serum and Rh_o (D) Immune Globulin. Initial objectives include the mechanism(s) of action and optimum conditions of production.

Methods Employed: Immunological, serological and physicochemical methods were used.

Major Findings: a) Anti-lymphocytic Sera: 1) Anti-lymphocytic sera effectively reduced the antibody plaque forming response in vitro. Allogeneic mouse anti-lymphocytic sera exhibited strain specificity while rabbit anti-mouse lymphocytic sera (Xenogeneic) demonstrated primarily species specificity. Commercial horse anti-mouse lymphocytic sera demonstrated only species specificity. Normal mouse sera, from the strains used, exhibited an antilytic property, apparently not antibody in nature. Strain differences in antilytic activity of sera and in peak plaque forming response were demonstrated. 2) Studies of a soluble extract from thymic tissue have been initiated.

b) Rh_o (D) Immune Globulin. Twenty-two laboratories performed in vitro potency tests of the Rh_o (D) Immune Globulin Reference, Lot 2 and data obtained by three different test methods have been summarized. Additional data were collected on the effects of commercial bovine and human albumin preparations on the radioassay; differences in the physicochemical characteristics of these albumins were demonstrated by immunological and serological methods. The bovine albumin preparation in use in this laboratory inhibits the non-specific binding of immunoglobulin to Rh negative erythrocytes in the radioassay.

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. This project fulfills these needs for antilymphocytic serum and Rh_o (D) Immune Globulin.

Proposed Course of Projects: This is a continuing project.

Honors and Awards: None

Publications: Paul, E.B. and Esposito, V.M.: Single dilution radio-immune assay for determination of anti-Rh_o (D) immune globulin. *Biochem. Biophys. Res. Comm.* 40:1380, 1970.

Esposito, V.M., Paul, E.B., and Seligmann, E.B., Jr.: *In vitro* potency results from a collaborative study of Rh_o (D) immune globulin reference preparation. In Press.

Serial No. (NIH-DBS) 177
1. Blood & Blood Products
2. Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Tissue Typing of the Human Histocompatibility (HL-A) Antigens

Previous Serial Number: Same

Principal Investigator: Vito M. Esposito, Ph.D.

Other Investigator: Mildred L. Woodward, B.S.

Man Years:
Total: 1.0
Professional: 1.0
Other: 0

Patient Days: None

Project Description:

Objectives: The overall objective is to develop standards for tissue typing of the human histocompatibility antigens. Initial objectives are: 1) development of testing methods, 2) establishment of a reference serum panel, and 3) establishment of a reference cell panel of known specificity.

Methods Employed: Serological, immunological, and physicochemical techniques were used.

Major Findings: a) A serum panel has been established through the cooperation of the Serum Bank of NIAID and additional serum will be added as the need arises.

b) A cytotoxicity test has been developed for typing of the human histocompatibility antigens and other techniques are being evaluated.

c) Proposed additional standards for Leucocyte - Platelet Typing Serum have been compiled.

d) A leucocyte cell panel of known specificity is being established.

Significance to Bio-Medical Research and the Program of the Division: Laboratory of Blood and Blood Products is responsible for control of the safety, purity, and potency of products derived from blood. This project is a part of that responsibility in that the objective is the formulation of standards for tissue typing sera.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: None

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Contracts

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LCA

LP

LVR

LVI

Adjuncts to OD

Contracts

Annual Report of the
Laboratory of Control Activities
Division of Biologics Standards
July 1, 1970 through June 30, 1971

I. Mission

The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity and potency. The Laboratory is composed of three sections: Analytical Chemistry, Control Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the approximate number tested by the Laboratory is 300 for sterility, 200 for safety, 100 for potency, 100 for chemical, 50 for pyrogen and 40 for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Research is conducted on development and modification of control tests on biological products.

(b) To develop, maintain and distribute biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with published standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) In additon to the above, the Laboratory shares responsibility with the other Laboratories in the Division in developing standards and regulations, performing inspections, reviewing license applications and maintaining a technical liaison with the World Health Organization, other national control agencies and recognized authorities in the field of biological products.

II. Product Testing

Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serve as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Control Test Section of the Laboratory of Control Activities provides regular cooperative pyrogen testing services to the Clinical Center Pharmacy, Blood Bank and Media Department and sterility testing for the Clinical Center Pharmacy and Blood Bank.

A summary of 12,509 tests performed by the laboratory during a 12 month period (April 1, 1970 through March 31, 1971) in making the determination that products meet prescribed standards is shown in Table 1.

TABLE 1

Samples Tested	Number of Tests Performed				
	Sterility	Safety	Potency	Purity	Total
Products for Release	5,065	932	909	1,407	8,313
Inspection	346	15	8	35	404
Cooperative Service	1,878	12	21	250	2,161
Reference and Standardization	52	0	449	36	537
Complaint	1	4	5	12	22
Research and Development	15	0	394	663	1,072
Totals	7,357	963	1,786	2,403	12,509

III. Standard Preparations

An integral part of the system of manufacture and control of biological products is the application and use of standard preparations. By the use of such standards, potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the laboratory to provide these standard and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of standards and references it was necessary to prepare and calibrate during the past year the following lots of materials: 14 antitoxins, 3 serums, 3 vaccines, 110 miscellaneous preparations. In addition to these materials prepared by the laboratory the following lots of materials were purchased from commercial sources and finished by the laboratory: 3 virus vaccines, 6 bacterial vaccines, 4 skin test antigens, 2 blood typing sera, and 1 blood group substance. A total of 537 tests were required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted _in vivo_ and _in vitro_ tests.

The technique for freeze-drying is a key procedure applied in the preparation of standard and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year.

Cultures	2,219	ampules
Serums	2,016	"
Vaccines	1,133	"
Viruses	1,450	"
Tuberculin	3,196	"
Plasma	250	"
Blood Group Substance	501	"
Toxin	29	"
Total	10,794	"

The following standard and reference preparations, consisting of a total of 1,022 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U.S. licenses and qualified foreign laboratories.

Australia Antigen	624
Antitoxins	456
Serums	12,387
Vaccines	2,218
Toxins	626
Bacterial & Viral Cultures	323
Allergens	457
Viruses	1,042

Opacity Standard	61
Globulins	125
Blood Products	1,099
Miscellaneous	53
Total	19,471

Approximately 120 different standards, references, and related control preparations are maintained for distribution.

IV. Related Activities

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) Members of the professional staff of the laboratory were members of 61 committees for the review of product and establishment license applications submitted to the Division.

(b) The laboratory staff participated in 63 of the on-site inspections required to be performed annually on all licensed establishments. Approximately 66 man days of work were consumed during the year in this effort.

V. Collaborative Studies with the World Health Organization and FDA.

The Laboratory of Control Activities works closely with the biological standardization program of the World Health Organization. During the past two years it has participated in collaborative studies on the Proposed International Plain and Adsorbed Tetanus and Diphtheria Toxoid Preparations.

The Chief of the Laboratory has been a member of the FDA Task Force on Use of Antibiotics in Animal Feeds and a member of the U.S.P. Advisory Panel on Microbiological Attributes and Procedures.

Periodically the International Reference Preparation for Rabies Vaccine (prepared by the Laboratory of Control Activities in 1963) is tested for potency by the laboratory in order to evaluate its stability. The Chief of the Laboratory served as a consultant to the World Health Organization in Geneva, Switzerland, and participated in the preparation of drafts of the International Minimum Requirements for Snake Antivenins and also served on the WHO Expert Committee on Biological Standardization.

VI. Research and Development

While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by laboratory personnel during this fiscal year.

(a) Opacity Standard: The U.S. Opacity Standard used in the standardization of cell concentrations of bacterial vaccines and of bacterial challenge suspensions used in potency tests is composed of finely divided glass particles suspended in water. During use and storage the opacity of the standard gradually lessens due to changes in particle size and adherence of particles to the walls of the container. Another undesirable feature is the difficulty of reproducing exactly the properties from lot to lot. For example the present international preparation differs from all previous international preparations as well as from the U.S. Standard. This is causing serious problems in laboratories throughout the world.

Tubes have been prepared with resin containing a homogenous suspension of particles of titanium dioxide. It was discovered that a concentration of the particles which yields light transmission equal to the U.S. Opacity Standard when compared with a nephelometer does not appear the same when compared by the unaided eye. Two sets of tubes have been prepared, one being equal to the Standard by instrument and the other by eye. Stability studies are in progress to determine the practicality of this type of solid containing static particles for estimating concentrations of bacterial cells in a liquid.

(b) Pseudomonas Vaccine: Studies in collaboration with the Laboratory of Bacterial Products have continued to provide basic data relative to the development of a Pseudomonas Vaccine. Studies have been made on the characteristics of several strains of the organism in relation to vaccine production, testing and use.

(c) Smallpox Vaccine: A broad program related to controlling the purity and potency of smallpox vaccine is continuing. A collaborative study with a large manufacturer of smallpox vaccine relative to the control of potency of vaccine with varied volumes of inoculum on the chorioallantoic membrane (CAM) of chicken eggs was carried out. The results of the study showed that licensed manufacturers of smallpox vaccine could use either 0.1 ml. or 0.2 ml. inoculum when testing vaccine potency by the CAM procedure.

(d) Sterility Testing: Several aspects of sterility testing are being studied with the object of improving the validity of sterility tests and reducing the time, volume of medium and cost involved in performing each test. New and modified media are being studied using selected microorganisms having peculiar growth characteristics. The use of membrane filtration for testing live virus vaccines containing antibiotics is being studied. An improved cutter for dividing the membrane in two exactly equal portions was developed.

(e) Tuberculin: Three collaborative studies with CDC have been conducted to study the stabilizing properties of Tween 80 in dilute solutions of tuberculin and to obtain a better understanding of the relationships between tests in sensitized guinea pigs and results of clinical use in man. As a result of these studies CDC is now distributing Tuberculin, PPD at the 5 T.U. strength which contains 5 ppm of Tween 80. Clinical studies are in progress with portions of large master batches of PPD produced by three manufacturers. The objective of these studies is to characterize these batches in order to evaluate better the meaning of reactions obtained in different populations. A large lot of the atypical mycobacterial strain, Battey, was prepared and freeze-dried for use as the U.S. Standard and for distribution from CDC for clinical testing. Studies on all aspects of tuberculins are being pursued actively.

(f) Typhus Vaccine: Vaccine prepared in a series of four two-fold levels of activity have been obtained under contract with a licensed manufacturer. The four preparations have been tested in laboratory studies for safety, potency and purity. The data are being assembled to be submitted as an application for clinical studies as an investigational new drug. It is planned to study the response in man to different levels of vaccine potency in order to establish the optinum potency for Typhus Vaccine. Modifications to the animal potency test are being developed in order to improve the sensitivity of that test. The project on Typhus Vaccine is being conducted in collaboration with the Laboratory of Virology and Rickettsiology.

Serial No. DBS 29
1. Control Activities
2. Analytical Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of methods for the chemical analysis of biological products.

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: J. Richard Miller
Roscoe M. Wheeler

Cooperating Units: None

Man Years

Total: 2.25
Professional: 1.25
Other: 1.0

Project Description:

Objectives: To develop new methods of chemical analysis of biological products or to modify existing methods, thus facilitating the study of chemical problems in the control of and research on biologicals.

Methods Employed: Standard methods of chemical analysis are used, evaluated, or modified as required for the material being analyzed, and new methods are developed.

Major Findings: It has been concluded that the electrolytic method for assaying residual moisture in freeze-dried biological products is less reliable than the gravimetric method. The electrolytic method is more subject to the introduction of error and does not save time. In the long run, many more samples may be assayed by the gravimetric method than is possible with the electrolytic method.

An extensive program is now under way to analyze dried biologicals by the traditional gravimetric method. This program will be expanded to investigate the possibility of using a micro gravimetric method or other available methods such as gas chromatography, Karl Fischer, and infrared spectroscopy for determining of moisture in dried biologicals.

Significance to Bio-medical Research and the Program of the Division: The significance of this work lies in providing increased knowledge of the

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chemical composition of biologicals which can be of value in research, and in surveillance of these products for the protection of the public.

Proposed Course of Project: The project will be continued with the addition of the following programs:

1) Expansion of the study of the amount of mercury assayed in all biologicals which have the mercurial preservative thimerosal (Merthiolate, Lilly) by a method developed in the Analytical Chemistry Section. These biological samples will be assayed through the entire dating period to determine if there is any change in the thimerosal level during storage. In addition, work will be initiated on developing methods or modifying existing methods for the thimerosal assay.

2) A program will be initiated to analyze L-asparaginase by the standard assay method, automated (Auto-Analyzer) method, and ion-selective electrode method and correlate the results obtained by these methods.

3) Work will be continued on the development of a method to measure alum and aluminum phosphate in biologicals by atomic absorption spectrophotometry.

Honors and Awards: None

Publications: None

Serial No. DBS 103
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Coccidioidin

Previous Serial Number: 36

Prinicpal Investigators: Vernon J. Fuller, Ph.D.
Edward B. Seligmann Jr., Ph.D.
Sotiros D. Chaparas, Ph.D.

Other Investigator: Ruben E. Marquina

Cooperating Units: Laboratory of Bacterial Products

Man Years

Total: 0.50
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To develop suitable laboratory procedures and reference preparations for use in the potency testing of coccidioidin.

Methods Employed: Guinea pigs were subjected to sensitization regimes of coccidioidin alone, spores alone, spores plus mycobacteria, spores and coccidioidin and, spores, coccidioidin, and mycobacteria. Skin testing was done with homologous and heterologous substances.

Major Findings: The level of sensitization varied depending on the combination of ingredients in the sensitizing preparation. Coccidioidin emulsions with spores, coccidioidin, and mycobacteria was the most effective combination for sensitization.

Significance to Bio-medical Research and the Program of the Division: The results emphasize that variations in the composition of ingredients used for sensitization to coccidioidin can cause marked differences in the level of reactivity of the guinea pig to reagents. Such factors must be taken into consideration in the development of a potency assay for coccidioidin.

Proposed Course of Project: Further studies will be made to elaborate the effects of dose of the substances in the sensitizing mixtures.

Two contracts were initiated; one with the University of California, Berkeley and the other with the University of California, Davis. Materials prepared by the contractors will be used in DBS studies.

Honors and Awards: None

Publications: None

Serial No. DBS 144
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: *Clostridium botulinum* Toxin and Antitoxin

Previous Serial Number: Same

Principal Investigator: Lee J. Stevan, M.S.

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: None

Man Years

Total: 1.0
Professional: 0.75
Other: 0

Project Description:

Objectives: To establish the relationship of various nutritional components of the growth medium to the biochemical structure of toxins of various strains of *Clostridium botulinum* types A, B, C, and E.

Methods Employed: Techniques of immunochemistry, fractionation procedures, biochemistry and bacteriology.

Major Findings: *C. botulinum* will grow and produce toxin in complex media with a number of different carbohydrates. Sporulation is stimulated with those carbohydrates which slow the growth rate of the organism. Carbohydrates which greatly stimulate the growth rate of the organism inhibit spore formation.

Ethylenediamine tetraacetate does not affect growth rate but reduces toxin production 100-fold and eliminates sporulation. Currently the protein differences between strains are being evaluated and antisera produced to the major protein fractions.

Significance to Bio-medical Research and the Program of the Division: This program should help explain the reasons why some lots of test toxins are inadequate. The potency testing of botulism antitoxins could be supplemented by the results of this project.

Proposed Course of Action: The results of this study will be evaluated and the project will be terminated at the end of the year.

Honors and Awards: None

Publications: None

Serial No. DBS 145
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Coral Snake Venom and Antivenin

Previous Serial Number: Same

Principle Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Lee J. Stevan, M.S.
William H. Berkeley

Cooperating Units: None

Man Years

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: Establishment of standards for coral snake antivenin. Study of the immunogenic properties of coral snake venoms from various species of snakes. Electrophoretic studies of coral snake venoms.

Methods Employed: Hyperimmunization of various species of animals. Venom neutralization tests. Immunoelectrophoresis, agar gel diffusion, acrylamide disc electrophoresis and agar gel electrophoresis.

Major Findings: Studies of the degree of cross neutralization of venom from various species of coral snakes by several commercial coral snake antivenins and some experimental specific antisera are in progress. Studies on venom from the Arizona coral snake (Micruroides euryxanthus) are being conducted with the small amount of available venom being the limiting factor.

Significance to Bio-medical Research and Program of the Division: As an outcome of work performed in the Laboratory, Wyeth Laboratories was licensed in September 1967 for Antivenin (Micrurus fulvius) which is effective against the venom of all coral snakes found in the United States except for Micruroides euryxanthus, the Arizona coral snake. Studies on cross-neutralization of venom from various species of coral snake are leading to an understanding of the extent of usefulness of coral snake antivenins for treatment of bites from coral snakes found throughout North, Central and South America.

Proposed Course of Project: Completion of work in progress on cross-neutralization and immunochemical properties of venoms from various species. Completion of work in progress on the *in vivo* effectiveness of antivenins in treating envenomation. Study of venom from the Arizona coral snake.

Honors and Awards: None

Publications: Stevan, L. J. and Seligmann Jr., E. B.: Agar-Gel and Acrylamide-Disc Electrophoresis of Coral Snake Venoms. Toxicon. Vol. 8, pages 11-14, 1970.

Serial No. DBS 147
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of an Improved Opacity Standard

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigator: Jane F. Farber

Cooperating Units: None

Man Years

Total: 0.15
Professional: 0.15
Other: 0

Project Description:

Objectives: 1) To prepare a stable solid opacity standard by suspending finely divided particles in a resin.

2) To standardize this preparation in terms of the current opacity standard which is an aqueous suspension of glass particles.

3) To determine the practicality of distributing a solid standard for use by manufacturers.

Methods Employed: Varying concentrations of titanium dioxide or glass particles are evenly suspended in viscous melted resin and allowed to harden in selected tubes. The concentration having the same light transmission as the U. S. Opacity Standard is then selected.

Major Findings: 1) Tubes of resin containing evenly suspended particles of titanium dioxide and having the same light transmission properties as the U. S. Opacity Standard have a different opacity when compared by means of the unaided eye.

2) Evaluation of a suspension of glass particles in resin established that this type of preparation would be unsatisfactory due to difficulties encountered in obtaining uniform dispersement of the glass particles in the resin.

3) A pilot lot of titanium dioxide in resin was prepared. Problems encountered in handling a relatively large volume of the heated resin were largely overcome. Tubes containing the suspension were prepared and evaluated for consistency. Uniformity was not perfect but variations are probably too slight to be of practical importance in actual use. Stability studies are in progress.

Significance to Bio-medical Research and the Program of the Division: The U.S. Opacity Standard is used to standardize the cell concentrations of bacterial vaccines and to achieve lot to lot consistency. The bacterial cell concentration of challenge doses used in potency tests are also adjusted with this standard. Because of the relative instability of the current aqueous suspension of glass particles a new lot must be prepared and distributed every few years. It is extremely difficult to reproduce the exact properties of a suspension of finely divided glass particles. Differences among lots and changes in a lot during use can lead to variation among lots of vaccine. As an example of the problems created by different lots of suspended glass opacity preparations the current lot of international reference differs significantly from any previous U.S. or international preparation and laboratories throughout the world are having difficulties in using it in the preparation of vaccines. Use of a permanent type of opacity standard such as a suspension of particles in a viscous resin or a solid plastic material of desired opacity would resolve many of these problems.

Proposed Course of Project: Continuation of stability studies and evaluation under laboratory conditions.

Honors and Awards: None

Publications: None

Serial No. DBS 150
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Replacement of the U.S. Standard Tetanus Antitoxin

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: None

Man Years

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To select a new lot of antitoxin that will prove suitable for use as a master dried U.S. Standard Tetanus Antitoxin, the supply of which is nearly depleted.

Methods Employed: The protective unitage relative to the master U.S. Standard Tetanus Antitoxin was determined by comparative neutralization tests in mice and guinea pigs.

Antitoxin characteristics such as avidity and heat stability were defined by the following methods using two toxins:

1) Comparison in mice of the L+ values obtained at test levels of L+/10, L+/100 and L+/1000.

2) Stability of toxin-antitoxin mixtures following periods of incubation at room temperature.

3) Comparison of the L+ values of glycerinated preparations stored at 37°C.

Major Findings: A dried preparation of tetanus antitoxin has been selected as the new master U.S. Standard Tetanus Antitoxin to replace the first master preparation which is now depleted. Through in vivo tests in this laboratory of several glycerinated lots in addition to the findings submitted by the licensed manufacturers following their evaluation of a glycerinated lot, the suitability of this preparation as the new master antitoxin has been established.

Proposed Course of Project: Project is completed.

Honors and Awards: None

Publications: Seligmann, E.B. Jr. and Farber, J.F. Freeze Drying and Residual Moisture. In press.

Serial No. DBS 153
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of a Reference Typhus Vaccine and an Improved Potency Test

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Otis L. Green
Harry T. Aylor
Elmer C. Russell

Cooperating Units: Laboratory of Virology and Rickettsiology

Man Years

Total: 0.25
Professional: 0.15
Other: 0.1

Project Description:

Objectives: Establishment of a suitable Reference Typhus Vaccine preparation. Development of an improved potency test for typhus vaccine.

Methods Employed: Standard potency test described in the Minimum Requirements for Typhus Vaccine and a variety of modifications including antigen extinction type tests.

Major Findings: No modifications of the animal potency test were found that were significantly better than the official test. However the test is not sensitive enough to differentiate between vaccines known to have a two-fold difference in antigen content. The complement fixation test is somewhat more sensitive, but not entirely satisfactory because of several very critical variables in the test.

Significance to Bio-medical Research and the Program of the Division: The effectiveness of present typhus vaccine has been questioned by the scientific community concerned with the use of typhus vaccine in the armed services. A reference vaccine would aid in assuring more consistency among production lots. In addition, the correlation between the animal test and effectiveness in man must be established.

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Proposed Course of Project: Under contract, typhus vaccine at four two-fold gradations in titer has been obtained and will be used in correlating laboratory assay procedures and clinical response.

Honors and Awards: None

Publications: None

Serial No. DBS 168
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Malayan Pit Viper Venom

Previous Serial Number: None

Principal Investigators: Lee J. Stevan, M.S.
D. L. Aronson, M.D. (LBBP)

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Blood and Blood Products

Man Years

Total: 0.15
Professional: 0.15
Other: 0

Project Description:

Objectives: To describe and evaluate the anti-coagulant and thrombolytic activity of Malayan pit viper (*Agkistrodon rhodostoma*) venom.

Methods Employed: Chromatography and electrophoresis, necessary tests used to evaluate the various venom fractions such as fibrinolysis, hemorrhagic activity and *in vitro* clotting of fibrinogen.

Major Findings: Little work was undertaken because of delays in the time schedule of the manufacturer developing the product. Preliminary work by DBS has been completed and further work is dependent upon the characteristics of the product to be manufactured.

Significance to Bio-medical Research and the Program of the Division: This investigation will lead to an understanding of the anti-coagulant and thrombolytic effects of Malayan pit viper venom. This understanding will in turn help the Division evaluate the potency and purity of any new drugs derived from this venom.

Proposed Course of Action: Development of suitable reference preparations and necessary supportive work after the manufacturer submits a product.

Honors and Awards: None

Publications: None

Serial No. DBS 169
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparison of Titrations in Monkey Kidney Tissue Cells and Chorioallantoic Membrane of Chick Embryos for the Potency Assay of Smallpox Vaccine.

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigator: Ruben E. Marquina

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To compare the potency of the U.S. Reference as determined by the chorioallantoic membrane method and the monkey kidney tissue cell (MKTC) method.

Methods Employed: The chorioallantoic membrane technique and MKTC method were employed in this study.

Major Findings: This project was held in abeyance during the current year due to priority given other studies.

Significance to Bio-medical Research and the Program of the Division: If the variability of the procedure can be brought under control, a third potency test method applicable to the standarization of smallpox vaccines may result from this study.

Proposed Course of Project: This study will be continued as time permits.

Honors and Awards: None

Publications: None

Serial No. DBS 170
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Standardization of Reference Blood Group Substance A and B.

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Jane F. Farber
Herman H. Hudson (LBBP)

Cooperating Units: Laboratory of Blood and Blood Products

Man Years

Total: 0.25
Professional: 0.25
Other: 0

Project Description:

Objectives: To select suitable blood group substances A and B preparations to replace the present Reference Blood Group Substance A and B upon depletion.

Methods Employed: The hemagglutination inhibition activity of several lots of candidate A & B substances was compared to that of the last reference preparation.

Major Findings: Preparations of A and B substances were compared by hemagglutination inhibition tests and suitable lots were selected for use as a reference. The relative activity of frozen and dried preparations was found to be approximately equal to that of a liquid preparation. The selected lots of A & B substances were combined to make a freeze-dried reference preparation containing 1 mg. of A and 1 mg. of B per milliliter.

Proposed Course of Project: Project is completed.

Honors and Awards: None

Publications: None

Serial No. DBS 178
1. Control Activities
2. Control Test
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron Microscopy of Vaccinia Virus Produced Under Conditions of Amino Acid Deprivation.

Previous Serial Number: Same

Principal Investigator: Edward A. Fitzgerald, Ph.D.

Other Investigator: Philip P. McGrath

Cooperating Units: Laboratory of Pathology

Man Years

Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: To investigate the morphology and development of vaccinia virions produced by host cells which are nutritionally deprived due to the omission of certain amino acids from the culture medium.

Methods Employed: The majority of virus particles produced under these conditions are not fully mature and only a small percentage are infectious. Electron microscopic examination of thin sections of infected cells which were fed with depleted medium show virus particles with apparent abnormalities in the structure of the outer coat. Further work will involve purification of the virus yield from these depleted cells using a sucrose density gradient. The gradient may then be fractionated and electron microscopy performed on certain of these fractions.

Major Findings: Only preliminary examinations of infected cells have been completed due to the time limitations imposed by a work of higher priority.

Significance to Bio-medical Research and the Program of the Division: This study will contribute to a further understanding of vaccinia virus morphogenesis and the effects of nutritional depletion on virus replication.

Proposed Course of Project: This study will be continued and expanded as time permits.

Honors and Awards: None

Publications: Ph.D. Thesis: Fitzgerald, E.A., Amino Acid Requirements in Vaccinia Virus Biosynthesis, Catholic University. Abstract published in
(1) Dissertation Abstracts International, Vol. XXXI, No. 5, 1970
(2) Bacteriological Proceedings. 1970, p. 196, No. V278.

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Serial No. DBS 180
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparison of Media Used in Sterility Testing

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigators: Edward B. Seligmann Jr., Ph.D.
Ruben E. Marquina

Cooperating Units: None

Man Years:

Total: 1.25
Professional: 0.75
Other: 0.5

Project Description:

Objectives: To compare the growth-promoting qualities of four media used in sterility testing. Fluid Thioglycollate medium; Fluid Sabouraud medium, pH 5.7; Fluid Sabouraud medium, pH 7.1 and Soybean-Casein Digest medium are being compared.

Methods Employed: Aliquots of serial dilutions of aerobic and anaerobic becterial and fungal cultures were inoculated into replicate tubes of the four test media. One set of tubes was incubated at 20-25°C. and the other at 30-32°C. for 10 days or longer depending upon the test organism.

Major Findings: In this study it was found that the optimum temperature for the 16 bacterial cultures studied was 31°C. and that under these conditions:

1) Fluid thioglycollate medium is the medium of choice for the propagation of both the aerobic and anaerobic organisms studied.

2) The modified Sabouraud medium and Soybean-Casein Digest medium are of equal value for growth of the organisms.

3) Fluid Sabouraud medium was of less value in propagating the bacteria. However, one must remember that Sabouraud's medium was developed for the

detection of mold, yeast, acidophilic bacteria and that the acid reaction of the medium is inhibitive to a large number of bacteria.

Significance to Bio-medical Research and the Program of the Division: The data obtained will aid in evaluating the media used in sterility testing by the Division and those used by other investigators.

Proposed Course of Project: The results of the fungi cultures are being tabulated and will be available shortly. The results of this study will be prepared for publication.

Honors and Awards: None

Publications: Russell, E. C., Farber, J. F., Seligmann, E. B. Jr.: Cutter for Membrane Filters Used in Sterility Testing. In press.

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Serial No. DBS 208
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of a Reference Tetanus Toxoid

Previous Serial Number: None

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Bacterial Products

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To select a reference tetanus toxoid that will prove most useful in determining the relative antigenic activity of commercially prepared toxoids. The following aspects of the problem will be investigated:

1) Relationship of *in vitro* and *in vivo* potency values.

2) Effect of varying amounts of mineral adjuvants combined with toxoids of varying potency.

3) Relationship of antigenic activity as determined in animals to the values obtained in human studies.

4) Determination of protective unitage against challenges of different toxins.

Methods Employed: The antigenic activity of fluid and adsorbed toxoids in guinea pigs and mice will be measured by the direct toxin challenge method and by neutralization test of serum antibodies. These findings will be correlated with activity determined in clinical studies. *In vivo* activity will be determined by the flocculation test.

Major Findings: The project is in the initial stages. Three toxoids have been selected for use, one of which is a fluid preparation, while the other two are adsorbed to different amounts of aluminum phosphate. Preliminary testing has begun on samples of these preparations.

Significance to Bio-medical Research and the Program of the Division: The precision of direct quantitative measurement of the potency of commercially prepared toxoids will be improved by comparative testing with a reference toxoid. The development of a laboratory test method which most closely correlates with the potency determined in human studies will enhance the accuracy of comparative testing.

Proposed Course of Project: To investigate all aspects of laboratory *in vivo* and *in vitro* test methods of measuring the immunizing activity of fluid and adsorbed tetanus toxoids and to correlate these findings with those determined in clinical studies.

Honors and Awards: None

Publications: None

Contracts

Adjuncts to OD

LVI

LVR

LP

Annual Report of the Laboratory of Pathology
Division of Biologics Standards
July 1, 1970 through June 30, 1971

The Laboratory of Pathology has a continuing program to study the pathogenesis of infectious diseases and immunologic processes using animal models. This program is designed to meet the overall needs of the Division in regard to the safety and efficacy of new biological products and also those which have been licensed for many years. The scope of the research activities of the laboratory has broadened considerably over the past several years. This is particularly so since the scientific personnel of the laboratory have interests which although broadly related to pathogenesis of infectious diseases, are related to biochemistry, virology, immunology, and histology as well as pathology.

The personnel of the laboratory has undergone considerable change within the past year. Dr. Ronald Clark, who had been in the laboratory for eight years and had essentially received most of his scientific training in that time (having received his doctorate while working here) left in December to join an academic faculty. Similarly, Dr. Deborah Carpenter, the only other fully trained pathologist decided to join the faculty of the Georgetown University School of Medicine. On the bright side, however, Dr. Stanley Singer who had left in 1969 to resume residency training, returned to the Division in August, 1970 and immediately resumed extremely productive studies. Dr. John Petricciani who has responsibilities for many contracts has become a member of the permanent staff. There is, however, a need for another individual with experience in pathology since requestsfor collaborative pathology studies are increasing rapidly.

Research Activities

The research aspects of neurovirulence testing of viral vaccines have become secondary to the testing procedures. However, past experience has shown that such tests must never be considered routine or the meticulous care needed to assure the safety of viral vaccines will be lost. This is particularly important at present, since consideration is being given to approval of production of Poliovirus Vaccine, Live, Oral using human cell strains as a substrate (Project Number 41, p.   ).

Recent discussions concerning the routine use of Smallpox Vaccine in the United States have led to the studies of the pathogenesis of various strains of vaccinia virus in monkeys. (Project Number 112, p.   )

Dr. Lorenz and other members of the staff of the Laboratory of Pathology and of Laboratory of Viral Immunology have in progress numerous studies of candidate agents of human hepatitis. The marmoset, Saguinus mystax, appears to be a useful animal model for studies of the pathogenesis of infectious (short incubation) hepatitis. To date, only the chimpanzee and man are susceptible to serum (long incubation) hepatitis. Studies of serum hepatitis in chimpanzees are being performed under Contract No. NIH 71-2040 with Hazleton Laboratories. These studies complement the work on infectious hepatitis in marmosets. (Project Number 133, p.).

Dr. Singer has resumed his investigations of the interaction of viruses, cells and mycoplasma and, in addition, with Dr. Hardegree of LBP, has studied the induction of interferon by some licensed biological products, namely some allergenic extracts and bacterial vaccines with no U. S. standard of potency. (Project Number 201 and 200, p.).

Dr. Petricciani in collaboration with investigators in NIMH has been studying various cell strains as to absence or presence of several enzyme activities and has shown that, by use of specific microorganisms, certain enzyme defects can be corrected. (Project No. 162, p.).

Dr. Shabo has continued and expanded studies begun by Dr. Ronald Clark who recently left NIH. These studies relate normal anatomic pathways of the central nervous system to barriers to viral infection and to immunologic mechanisms and suggest methods for allowing drugs and antiviral substances to enter the cerebrospinal fluid and perhaps the central nervous system tissue itself. (Project No. 202, p.).

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program as all other research activities is under the direction, supervision, and coordination of the Chief of the Laboratory.

Further, the Chief of the Laboratory has directed most of her own research efforts in the general field of the pathogenesis of infectious diseases with particular emphasis on oncogenic viruses. The pathogenesis of the particular tumors induced, the effect of altered immunologic mechanisms, and the interaction of these viruses on each other are all being studied. At the present time the possible oncogenicity of herpes viruses is of particular interest (Project No. 44, p.).

Collaborative (Contracts) Activities

The Laboratory of Pathology plays a very active role in the collaborative program of the Division, since most if not all contracts have aspects relating to pathogenesis and require pathologic interpretation. The Chief of the Laboratory of Pathology works very closely with the various project officers, the Contracts Operations Officer, and the Director, DBS in helping to plan and execute various projects and to review progress reports of those contracts already in existence. She also reviews histologic material from contract work as necessary. Furthermore, she serves as a liaison between Collaborative Program Directors of other Institutes, the Assistant Director of NIH for Collaborative Research, and the Division.

Dr. Petricciani has served as Project Officer for four contracts concerning the development of diploid cell strains from sub-human primates and another contract concerning survival of donor white blood cells in recipients. He also served as an Assistant Project Officer for one contract while Dr. Lorenz served as Assistant Project Officer for three contracts. Dr. Chisari serves as Assistant Project Officer for one contract.

Control Activities

During the past year, the Laboratory has performed neurovirulence tests on 28 lots of Poliovirus Vaccine, Live, Oral, for release as well as tested the Reference Vaccine on seven occasions. Fourteen lots of Measles Virus Vaccine have been tested. Eight lots of Rubella Virus Vaccine and one lot of Mumps Virus Vaccine have been tested. A total of 1685 monkeys have been used for this purpose. The safety of these vaccines has been well controlled.

One hundred and eighty-two vervet monkeys and 75 rhesus monkeys were sacrificed in order to remove kidneys for the Cell Biology Section, LVR. Three hundred and eighteen rabbits were similarly sacrificed.

The Histopathology Section produced 20,452 sections for light microscopy, 500 of which were of celloidin prepared material, 2300 sections for electron microscopic study, and 180 scanning electron microscopy negatives. Eight thousand photographic prints were made.

The Chief of the Laboratory has served as Chairman of seven license application review committees and on five other such committees. She has prepared regulatory standards for Poliovirus Vaccine, Live, Oral; Adenovirus Vaccine, Live, Oral; and L-Asparaginase. Two-hundred and thirty-five protocols have been reviewed prior to release as follows: Poliovirus Vaccine, Live, Oral: 68; Poliomyelitis Vaccine: 7; Live Attenuated Measles Virus Vaccine: 100; Mumps Virus Vaccine, Live: 21; Rubella Virus Vaccine: 39. Each permanent staff member has participated in the annual inspection program performing inspections at five establishments.

Serial No. DBS 41
1. Pathology
2. Animal Testing and
   Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Developmental Studies of Neurovirulence Testing on Poliovirus Vaccine, Live, Oral, in Monkeys

Previous Serial Number: 41

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Alan L. Shabo, M.D., LP-DBS
George Rusten, LP-DBS

Cooperating Units: Serology Section, LVI, screens monkey serums for freedom from antibody to poliovirus.

Man Years:
Total: 5.0
Professional: 0.4
Other: 4.6

Project Description:

Objectives: To develop information concerning the reproducibility and sensitivity of neurovirulence tests of Live Poliovirus Vaccines, to refine techniques used in the test to assure safety of the vaccine and to test the vaccines being produced on new cell substrates.

Methods Employed: Monkeys were inoculated with vaccine or dilutions thereof either intrathalamically, intraspinally, or intramuscularly and observed for evidence of developing paralysis over a period of 17-18 days. The monkeys were then sacrificed and the central nervous system examined histologically for evidence of poliomyelitis. In addition, tissues were saved for virus isolation attempts. These studies were performed as outlined in the PHS Regulations for Poliovirus Vaccine, Live, Oral.

Major Findings: The new neurovirulence reference vaccine which was selected based on replicate testing of several candidate vaccines both by neurovirulence tests prescribed for an acceptable seed virus [(Sec. 73.110 (b)(4)] and the test performed on all vaccine lots [Sec. 73.114(b)(1)] was evaluated further. Eight lots of vaccine which were produced on a new cell substrate, namely, human diploid cells have been tested in order to have assurance that the neurovirulence properties of the vaccine have not been altered by this new production method.

The collaborative study which was undertaken with other control laboratories has been completed and is awaiting publication.

In order to ensure the safety of Poliovirus Vaccine, Live, Oral, licensed for use in the United States, all lots of vaccine submitted for release have been tested. Twelve lots of Type 3 and eight of Type 1 have been tested as well as two lots of Type 2 vaccine. The newly selected Reference Vaccine has been tested six times.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to assure standardization of neurovirulence testing of poliovirus vaccines throughout the world, to assure the safety of such vaccines, and to improve testing procedures.

Proposed Course of Project: It is anticipated that collaborative studies to standardize safety testing of vaccines will be a continuing project. The present study is being prepared for publication.

Honors and Awards: Dr. Ruth L. Kirschstein served as a consultant to the World Health Organization on international requirements for Poliovirus Vaccine, Live, Oral, May 3-7, 1971.

Publications:

Kirschstein, R. L., and Palmer, A. E., A Primate Program Developed for the Testing of Biological Products. Proceedings of the Second Conference on Experimental Medicine and Surgery in Primates, S. Karger and Co., Switzerland (in press).

Serial No. DBS 44
1. Pathology
2. Animal Testing and
   Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Pathogenesis of Infectious Diseases

Previous Serial Number: 44

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators:
Francis Chisari, M.D., LP-DBS
Alan Rabson, M.D., NCI
Elizabeth Peters, LP-DBS
Stanley Singer, M.D., LP-DBS
Harold Baer, Ph.D., LBP-DBS
Carl Miller, D.V.M., LBP-DBS
Michael Barile, Ph.D., LBP-DBS
Mitchell Friedlander, M.D., LBP-DBS
Pinya Cohen, Ph.D., LBBP-DBS
Milton Brightman, Ph.D., NINDS
Carolyn Hardegree, M.D., LBP-DBS
John C. Feeley, Ph.D., LBP-DBS
L. F. Barker, M.D., LVI-DBS
A. W. Cheever, M.D., NIAID
Douglas Lorenz, Ph.D., LP-DBS
Michael Peterson, M.D., LVI-DBS
Robert Northrup, M.D., NIAID
Alan L. Shabo, M.D., LP-DBS

Cooperating Units:
Pathologic Anatomy Branch, NCI
Laboratory of Bacterial Products, DBS
Laboratory of Blood and Blood Products, DBS
Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS
Laboratory of Viral Immunology, DBS
Laboratory of Parasitic Diseases, NIAID
Laboratory of Clinical Investigations, NIAID

Man Years:
Total: 7.0
Professional: 1.8
Other: 5.2

Project Description:

Objectives: Experimental infectious diseases are studied as to pathogenesis in the hope that this may further elucidate the pathogenesis of infectious diseases in man and lead to their prevention.

Methods Employed: Infectious agents and related substances or substances causing an immunologic response are injected into a variety of animals when newborn or adult, and gross autopsies and histologic examinations are performed at timed intervals so that the development of the disease process and the pathologic changes may be studied.

Major Findings: (1) (With Drs. Rabson, Lorenz and Mrs. Peters) The pathogenesis of the Herpes group of viruses in newborn and adult hamsters, mice and rats after various routes of inoculation is being studied. Despite the association of "herpes-like particles" with the "Burkitt" type lymphoma, Herpes simplex and cytomegaloviruses have no effect on the lymphoreticular system. Attempts to alter infectivity are underway, using a variety of chemical and physical means. Other members of the Herpes group such as *Herpesvirus saimiri* are being studied also.

(2) (With Dr. Rabson and Mrs. Peters) Studies of the ability of the oncogenic viruses to "transform" hamster cells, monkey cells, and cells of other species in cell cultures are continuing. The cellular transformations have characteristics of malignancies and varying growth potential when inoculated into animals, depending on the species. Attempts to alter this growth potential by changing immune response and using other target organs are continuing.

(3) (With Dr. Cohen and Dr. Hardegree) Studies of the tumors occurring in mice after subcutaneous inoculation of components of oil adjuvant, either alone, in combination with other components or in combination with carcinogens are continuing as a part of the evaluation of the results obtained in the study carried out in Contract No. PH43-67-679.

(4) (With Dr. Singer and Dr. Barile) Studies of the interaction of cells and viruses as well as cells, viruses, and mycoplasma have been resumed since the return of the principal investigator to NIH.

(5) (With Dr. Barker) Studies of the pathogenesis of various strains of vaccinia virus in mice are still in progress.

(6) (With Dr. Cheever) A study of naturally occurring *Schistosoma mansoni* infection of African green monkeys indicates that in some animals the infection is widespread and causes severe cirrhosis of the liver and pneumonitis.

(7) (With Drs. Lorenz, Chisari, Barker, and Peterson) Studies of candidate agents of human infectious and serum hepatitis have been reported and are continuing as described in Project Number 133.

(8) (With Drs. Chisari, Northrup, Miller, and Feeley) Studies of the possible induction of cholera in monkeys and of the action of the toxin of *Vibrio cholerae* and its toxoid have just been initiated.

(9) (With Drs. Brightman, Shabo and Mrs. Peters) Studies of comparative oncogenic effect of SV40 and polyoma viruses inoculated intracerebrally are being studied at the ultrastructural level.

(10) (With Drs. Chisari, Friedlander, and Baer) The effects of poison ivy extracts on "skin islands" in which the drainage is controlled are being investigated.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to elucidate aspects of the pathogenesis of infectious diseases in animals so that these models may serve to help provide the means of prevention of such diseases in man.

Proposed Course of Project: It is expected that this program will expand in 1971-72, particularly since there will be several new staff members in the laboratory.

Honors and Awards: Dr. Ruth L. Kirschstein continues to serve on the Contract Review Committee, Breast Cancer Task Force, N.C.I.

Dr. Ruth L. Kirschstein continues to serve as a member of *Ad Hoc* Task Group for Interferon Research, NIAID.

Publications:

Cheever, A. W., Kirschstein, R. L., and Reardon, L. V., *Schistosoma mansoni* infection of presumed natural origin in *Cercopithecus* monkeys from Tanzania and Ethiopia, Bulletin of World Health Organization 42:486-490 (1970).

Lorenz, D., Barker, L., Stevens, D., Peterson, M., and Kirschstein, R., Hepatitis in the Marmoset, *Saguinus mystax*, Proc. Soc. Exp. Biol. Med. 135:348-354, 1970.

Serial No. DBS 45
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Histologic and Ultrastructural Development of the Central Nervous System and its Relationship to the Immunological Response

Previous Serial Number: 45

Principal Investigator: Ronald G. Clark, Ph.D.

Other Investigators: Philip McGrath, LP-DBS
Joyce Johnson, LP-DBS
Charles Manclark, Ph.D., LBP-DBS
Walter Stanley, Ph.D., LP-NIMH
Paul Albrecht, M.D., LVR-DBS
Thomas Milhorat, M.D., SN-NINDS
Sotiros Chaparas, Ph.D., LBP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS
Laboratory of Virology and Rickettsiology, DBS
Laboratory of Psychology, NIMH
Surgical Neurology Branch, NINDS

Man Years:
Total: 2.5
Professional: 1.5
Other: 1.0

Project Description:

Objectives: To ascertain the normal developmental processes in the nervous system of laboratory animals and to relate ultrastructural alterations of these developmental processes in experimental animals in response to immunologic stimulation.

Methods Employed: *In utero* and postnatal animals are sacrificed at timed intervals, and gross evaluation and histologic examinations are performed to determine the normal development of the central nervous system. The histologic evaluation involves both light and electron microscopy. Experimental animals are studied similarly, and microscopic as well as ultrastructural deviations from the normal are noted and evaluated.

Major Findings: (1) (With Dr. Albrecht) Normal rhesus monkeys, immunosuppressed monkeys, and monkeys in the early stages of experimental allergic encephalomyelitis were inoculated with measles virus in order to determine the neurotropic properties of this virus under varying immunological conditions in the monkey. The study of one strain of measles virus did not indicate neurotropism.

(2) (With Dr. Stanley) A very high incidence of hydrocephalus, over sixty percent, was found to exist in at least three supposedly normal purebred beagle colonies. A manuscript is in preparation presenting these findings. Dr. Clark is continuing this study at the University of Miami.

(3) (With Dr. Milhorat and Mr. McGrath) Experimental obstructive hydrocephalus was produced in the rhesus monkey, representing the first such experimental model to be developed for the study of this disease in primates. It was found that the ventricular system dilates quite rapidly upon complete ventricular obstruction. Cellular damage and cerebral edema occur within a few hours following such obstruction.

Significance to Bio-Medical Research and the Program of the Division: These studies are closely related to the problem of postvaccinal encephalitis and the production of vaccines in tissue from the central nervous system of animals and also may elucidate the causation of congenital abnormalities of the central nervous system.

Proposed Course of Project: The principal investigator has left the Division to take an academic position at the University of Miami Medical School and work in progress on this project at DBS has been completed by the end of this report year.

Honors and Awards: None

Publications:

Chaparas, S. D., Clark, R. G., Kies, M. W., and Hedrick, S. R., Transformation of lymphocytes from monkeys with experimental allergic encephalomyelitis, Res. Comm. Chem. Path. Pharm. (September 1970).

Clark, Ronald G., Milhorat, Thomas H., Stanley, Walter C., and DiChiro, Giovanni, Experimental pantopaque ventriculography. J. Neurosurg., (March 1971).

Clark, R. G., Milhorat, T. H., McGrath, P. P., Hammock, M. K., Light microscopic and ultrastructural changes obstructive in hydrocephalus in monkeys. Symposium on Cisternography and Hydrocephalus. Charles C. Thomas, Publisher, May 1971.

Serial No. DBS 112
1. Pathology
2. Animal Testing and
Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Developmental Studies and Control Testing of Attenuated Live Virus Vaccines

Previous Serial Number: 112

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators:
Alan L. Shabo, M.D., LP-DBS
Harry Meyer, M.D., LVI-DBS
Paul Parkman, M.D., LVI-DBS
Nicola Tauraso, M.D., LVR-DBS
Edward B. Seligmann, Ph.D., LCA-DBS
L. F. Barker, M.D., LVI-DBS
Paul Albrecht, M.D., LVR-DBS

Cooperating Units:
Laboratory of Viral Immunology
Laboratory of Virology and Rickettsiology
Laboratory of Control Activities

Man Years:
Total: 3.0
Professional: 0.4
Other: 2.6

Project Description:

Objectives: To gain information concerning the pathogenesis of viral encephalitides in monkeys so that safety tests for attenuated live virus vaccines can be developed and the safety of the vaccines assured.

Methods Employed: Rhesus monkeys are inoculated with both wild and attenuated virus preparations, using various routes of administration depending on the mode of action of the particular virus. Animals are observed for evidence of disease, and specimens of blood and various secretions are taken for evidence of virus multiplication. Uninoculated animals may be placed in cages with inoculated animals or in the same room in order to study transmissibility of the wild or attenuated infection. At the end of the observation period which depends on the virus and the route of inoculation, all animals are bled to determine antibody levels. The monkeys are then sacrificed, and various organs are assayed for presence of virus.

All organs from each animal are examined histologically for evidence of abnormality.

Major Findings: (1) (With LVR and LCA) Studies of Yellow Fever Vaccine (17D strain) after removal of the contaminating avian leukosis virus indicate that its neurovirulence has not been altered and that it is immunogenic for monkeys. This indicates that the vaccine has retained its desirable properties during the processing to remove the adventitious virus. Studies of other strains are in progress.

(2) (With LVI) Studies of the pathogenesis of rubella virus infection in monkeys are complete. On the basis of this study, the neurovirulence safety tests for rubella virus vaccine have been developed and licenses were granted to three manufacturers. Eight lots of rubella vaccine have been tested.

(3) (With LVI) Studies on the pathogenesis of various vaccinia virus strains in rhesus monkeys are in progress.

(4) (With LVR) Attempts to adapt human measles virus to non-immune primate hosts are continuing.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to develop information which will allow control testing of new viral vaccines and also some of the older vaccines to be more meaningful and assure safety of the product.

Proposed Course of Project: It is expected that this program continue during the next years as more new vaccines are developed or new vaccine strains are proposed for use.

Honors and Awards: None

Publications: None

Serial No. DBS 133
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Marmosets and Studies of Hepatitis in Sub-human Primates

Previous Serial Number: 133

Principal Investigator: Douglas Lorenz, Ph.D., LP-DBS

Other Investigators:
Ruth L. Kirschstein, M.D., LP-DBS
Francis Chisari, M.D., LP-DBS
Richard Washington, LP-DBS
Lewellys Barker, M.D., LVI-DBS
Michael Peterson, M.D., LVI-DBS

Cooperating Units: Laboratory of Viral Immunology, DBS

Man Years:
Total: 2.5
Professional: 1.0
Other: 1.5

Project Description:

Objectives: (1) to maintain marmosets under laboratory conditions, (2) to describe the microbiology of these animals, and (3) to use marmosets, as well as other sub-human primates, to study human hepatitis.

Methods Employed: Ten pairs of *Saguinus mystax* and their offspring are being maintained under routine laboratory conditions. Fifty *S. oedipus* and 30 *S. nigricollis* were acquired and were maintained by routine procedures. *Cercopithecus aethiops* and *Erythrocebus patas* were also used. The various species were isolated before and during experimentation. Classical diagnostic laboratory procedures were employed.

Major Findings: (1) *Colony maintenance*: Ten pairs of *Saguinus mystax* which had been segregated two years ago are being maintained as breeders. Thirteen offspring have survived in the last year and a half. Three pairs of laboratory bred animals have been established. All of these animals have adjusted to standard primate maintenance conditions.

Of the three species of marmosets which have been studied, S. oedipus, the cotton top marmoset, has been found to be the most hardy and to require the least care upon receipt from suppliers. This species is the most readily available. S. nigricollis, the white-lip marmoset, are too small to be considered a standard laboratory primate. The parasite burdens of the three species have been found to be about equal. S. mystax is the species most difficult to acquire.

(2) Cell Culture and Virology: Cell cultures have been established and maintained for extended periods of time from all three species of marmosets. Adventitious agents were not found after prolonged maintenance of the cultures. The cells do not tend to transform spontaneously.

One agent, derived from Herpesvirus saimiri, produces a fatal proliferation of lymphoid cells in S. mystax. Kidney cells from infected animals can be cultured for extended periods of time and continue to produce a herpes virus.

Hepatitis materials applied to marmoset cell cultures have not demonstrated any consistent effect on the cells.

(3) Hepatitis Studies: A major study using Saguinus oedipus has been completed. Four coded serum specimens from hepatitis patients were supplied by the Armed Forces Epidemiology Board, Subcommittee on Liver Disease. Each of the four specimens were inoculated intramuscularly into six marmosets. The animals were maintained for 10 weeks. The concentration of their serum glutamic-pyruvic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT) were determined weekly and a liver biopsy was examined weekly also. No diagnostic pattern was recognized in any of the cotton top marmosets. Subsequently, Deinhardt's hepatitis agent was inoculated intramuscularly into all of the marmosets. Elevated values for SGPT and SGOT occurred in many animals after four weeks. Only one group of marmosets inoculated with a human serum was totally refractive to Deinhardt's agent. When the code was broken it was realized that the refractive group was the one inoculated with a hepatitis agent which could be passed in human volunteers. Two other groups had been inoculated with normal human sera and the fourth group had been inoculated with serum which would not infect other volunteers, but would infect marmosets according to other investigators. It was concluded from this experiment that the cotton top marmoset does not react consistently to hepatitis agents and, therefore, was dropped as an experimental animal in these studies.

Cercopithecus aethiops, African green monkeys, and Erythrocebus patas, patas monkeys, were inoculated with sera containing hepatitis associated antigen (HAA) and with purified HAA. In addition, some of the animals were treated with the immune suppressant, cyclophosphamide. Tests for HAA, SGOT, and SGPT generally were conducted weekly for 10 weeks. Liver biopsies were also examined weekly. To date none of these animals have shown consistent signs of infection.

Significance to Bio-Medical Research and the Program of the Division: A closed colony of the small primates, *Saguinus mystax*, which are susceptible to some human hepatitis agents and a herpes virus which produces a leukemia in the animals provides DBS with an excellent model to study the pathology of these two diseases, as well as their immunologic or therapeutic control. An understanding of techniques for cultivation of the cells from marmosets allows *in vitro* studies. The hepatitis studies provide a capability for conducting evaluation of or exploratory investigations of possible hepatitis reagents.

Proposed Course of Project: The major effort will be directed toward hepatitis experiments in primates. The breeding colony of marmosets will be continued but limited to productive wild animals and laboratory bred animals. Cell cultures from marmosets will be investigated as a means of identifying hepatitis agents and as a means of investigating tumor inducing agents.

Honors and Awards: None

Publications:

Lorenz, D., L. Barker, D. Stevens, M. Peterson, and R. Kirschstein, 1970, Hepatitis in the Marmoset, *Saguinus mystax*. Proc. Soc. Exptl. Biol. Med. 135:348-354.

Baer, H. and D. Lorenz, 1970, The Induction of Immediate and Delayed Sensitivity in the Marmoset. Proc. Soc. Exptl. Biol. Med. 134:410-412.

Serial No. DBS 161
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chromosomal Effects of in utero Viral Infections on Sub-human Primates

Previous Serial Number: 161

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Ruth L. Kirschstein, M.D.

Cooperating Units: None

Man Years:
    Total: 0.65
    Professional: 0.40
    Other: 0.20

Project Description:

Objectives: The relationship of early intrauterine infection by certain viruses to various congenital abnormalities is well documented. The possible role of other viruses in the development of an abnormal karyotype in the fetus of a sub-human system is being investigated.

Methods Employed: Multiple skin biopsies and one unilateral nephrectomy were performed to establish cell cultures from newborn stump-tailed monkeys (*Macaca speciosa*) for the purpose of viral susceptibility screening and baseline cytogenetics.

Major Findings: All of the cell cultures established in this study developed a CPE similar to that caused by Foamy virus 2-3 weeks after being initiated.

Significance to Bio-Medical Research and the Program of the Division: These studies may provide evidence for the viral etiology of an abnormal karyotype in a sub-human primate system with possible significance for man.

Proposed Course of Project: These studies have been terminated because of the latent agent found in the primary cell cultures.

Honors and Awards: None

Publications: None

Serial No. DBS 162
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effect of Bacterial Viruses on Animal Virus Infectivity and on Animal Cells

Previous Serial Number: 162

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Carl R. Merril, M.D.

Cooperating Units: Laboratory of General and Comparative Biochemistry, NIMH

Man Years:
Total: 1.25
Professional: 1.25
Other: 0

Project Description:

Objectives: The uptake and subsequent fate of bacteriophage in mammalian cell cultures is being studied.

Methods Employed: Both intact phage and extracted nucleic acid are inoculated onto mammalian cell cultures which are then observed for morphologic and biochemical changes.

Major Findings: Preliminary data suggests that there are biochemical effects of bacteriophage on mammalian cells in culture.

Significance to Bio-Medical Research and the Program of the Division: These studies may provide information on the possible use of components of bacterial viruses as anti-animal virus agents and data on their biologic potential in genetic therapy in animal cells is being assessed.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: None

Publications: None

Serial No. DBS 200
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Interferon Production by Allergenic Products

Previous Serial Number: None

Principal Investigators: Stanley H. Singer, M.D.
M. Carolyn Hardegree, M.D.

Other Investigators: Peter J. Geroni, Sc.D.

Cooperating Units: Laboratory of Bacterial Products, DBS
Virus and Rickettsia Division, Ft. Detrick

Man Years:
Total: 1
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To determine whether or not bacterial vaccines with "No U. S. Standard of Potency" and allergenic extracts are capable of inducing interferon.

Methods Employed: Mice are injected by various routes with the above described biologics. Interferon titers from pooled sera are determined using standard virus yield reduction assays in primary mouse embryo cell cultures. The antiviral activity achieved in mice is determined by a live virus challenge using a variety of routes.

Major Findings: High levels of circulating interferon are induced by certain of the bacterial vaccines and allergenic extracts. Interferon production occurs after inoculation by a variety of routes. The induced interferon protects mice against an otherwise lethal virus challenge.

Significance to Bio-Medical Research and the Program of the Division: It has been determined that certain licensed biologics have the capacity to induce interferon in mice and to protect them against a viral challenge under

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defined experimental conditions. It is possible that this activity may be associated with the clinical effectiveness of this type of product in man.

Proposed Course of Project: These studies will continue during the next fiscal year.

Honors and Awards: None

Publications:

Singer, S. H. and Hardegree, M. C. Induction of Interferon by Bacterial Vaccines and Allergenic Extracts. Journal of Allergy (In Press).

Serial No. DBS 201
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mycoplasma-Virus Interaction

Previous Serial Number: None

Principal Investigator: Stanley H. Singer, M.D.

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
Michael Barile, Ph. D., LBP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS

Man Years:
Total: 1
Professional: 0.5
Other: 0.5

Project Description:

Objectives: The effects of dual infection with a mycoplasma and a virus are being studied in *in vitro* and *in vivo* situations.

Methods Employed: Chronic mycoplasma infection is established and then cultures or animals are super-infected with viruses. Adsorption, penetration, replication, and pathogenicity of the virus are then determined.

Major Findings: We have previously demonstrated that contamination of hamster cell cultures by mycoplasma can influence interferon production and, thus, virus production. We have extended these findings *in vitro* to other species using both continuous cell lines and primary cell cultures. We have demonstrated also that the final result of virus multiplication is not only a function of the particular mycoplasma, the particular virus, and the particular cell species used, but also depends on whether the cell culture is primary or continuous. In addition, it appears that chronic mycoplasma infection *in vitro* can alter the pathogenicity of an infecting virus.

Significance to Bio-Medical Research and the Program of the Division: Mycoplasma can be isolated from a substantial proportion of the population. Since mycoplasma can alter host responses to virus infections it is possible that carriers of mycoplasma may be more susceptible to certain virus infections. These studies may lend insight into why there is such individual variation in the pathogenic expression of a virus.

Proposed Course of Project: These studies will continue during the next fiscal year.

Honors and Awards: Dr. Singer is an invited participant in a round table discussion on selected topics of mycoplasmology at the meeting of the American Society of Microbiology May 1971.

Publications: None

Serial No. DBS 202
1. Pathology
2. Animal Testing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Histologic and Ultrastructural Studies on the Central Nervous System and Eye with the Identification of Membrane Systems which may be Immunologically Important

Previous Serial Number: None

Principal Investigator: Alan L. Shabo, M.D.

Other Investigators: Dr. David Maxwell, U.C.L.A. School of Medicine
Dr. Thomas Reese, LNNS-NINDS
Dr. John Petricciani, LP-DBS
Dr. Ruth Kirschstein, LP-DBS

Cooperating Units: U.C.L.A. School of Medicine
Laboratory of Neuropathology & Neuroanatomical Sciences, NINDS

Man Years:
Total: 1.5
Professional: 1.0
Other: 0.5

Project Description:

Objectives: To determine the anatomic relationships of the normal CSF and aqueous humor pathways following the introduction of tracer substances.

Methods Employed: Animal tissues are prepared by special techniques for light microscopic and ultrastructural evaluation of normal structure. In experimental animals, the fate of tracer substances such as peroxidase and ferritin introduced into the CSF and aqueous pathways is determined over a period of time. A method is being developed to tag antibody to electron-dense tracers.

Major Findings: (1) (With Dr. Maxwell) The arachnoid villus in the CSF pathway and the trabecular meshwork in the aqueous pathway are analogous structures structurally and functionally. Arachnoid and trabecular meshwork cells are latent macrophages which remove tracer substances from their respective pathways by phagocytosis.

(2) (With Dr. Reese and Dr. Maxwell) The endothelial cells of the dural venous sinus (CSF pathway) and Schlemm's canal (aqueous pathway) apparently possess tight junctional complexes which restrict the passage of large molecules from the CSF and aqueous humor into the systemic blood and, thus, may represent immunologically significant barriers. This observation will require further verification.

(3) (With Dr. Petricciani and Dr. Kirschstein) Monkeys were inoculated intraspinally with poliovirus. Anti-poliovirus antibody was obtained and conjugated to peroxidase. The tissue was then incubated in the antibody-peroxidase conjugate. This project was recently initiated and results are not yet available.

Significance to Bio-Medical Research and the Program of the Division: These studies relate to the anatomic pathways through which biological products, drugs, and microorganisms may enter and leave the CNS and the eye. Additionally, since arachnoid and trabecular meshwork cells probably represent the reticulo-endothelial system for the CNS and the eye, observations with tracer substances provide a means of examining the cellular response of these tissues. Finally, the apparent restriction of the passage of large molecules from the CNS and the eye into the systemic blood may immunologically relate to the etiologies of post-vaccinal, allergic encephalomyelitis and allergic uveitis.

Proposed Course of Project: This study will progress with increased emphasis on an examination of the normal membrane pathways with immunologic substances.

Honors and Awards: Invited to participate in International Symposium on the CSF for the XIV Congress of Latin American Neurosurgery, March 10-13, Punta del Este, Uruguay.

Publications:

Shabo, A. L. and Maxwell, D. S. The fine structure of the arachnoid villus under normal and experimental conditions. Symposium on CSF - XIV Congress of Latin American Neurosurgery. (In press)

Serial No. DBS 203
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Scanning and Transmission electron microscopy of materials used in testing biological products

Previous Serial Number: None

Principal Investigator: Philip P. McGrath

Other Investigators: Ronald G. Clark, Ph. D., University of Miami
John B. Ewell, LP-DBS
Hope E. Hopps, M.S., LVI-DBS
Sotiros Chaparas, Ph. D., LBP-DBS
Vito M. Esposito, Ph. D., LBBP-DBS

Cooperating Units: University of Miami School of Medicine, Miami, Florida
Laboratory of Viral Immunology, DBS
Laboratory of Bacterial Products, DBS
Laboratory of Blood and Blood Products, DBS

Man Years:
Total: 2.3
Professional: 1.0
Other: 1.3

Project Description:

Objectives: To relate surface and intracellular ultrastructural changes in tissues and cells to infection with microorganisms or response to immunological reagents.

Methods Employed: Samples to be examined are prepared for scanning and transmission electron microscopy. The surface structures of normal samples are compared with experimental samples to determine changes induced by the particular experimental manipulations. These results from the scanning electron microscope are compared with light microscopic and transmission electron microscopic findings and evaluated as to effects of the agents used in the experimental system.

Major Findings: (1) (With Dr. Clark and Mr. Ewell) The surface structures of the cells lining the ventricles of the brain were studied. The choroid plexus lateral wall ependyma surfaces of normal and hydrocephalic monkeys were compared. Some edema was observed in the choroid plexus ependyma in hydrocephalic monkeys. A large epiplexus macrophage was found associated with the choroid plexus ependyma of both normal and hydrocephalic monkeys.

(2) (With Mrs. Hopps) Cell cultures infected with rubella virus are being examined in the scanning and transmission electron microscopes to determine cell surface changes which can be correlated with virus growth. This study has recently been initiated.

(3) (With Dr. Chaparas) The morphologic characterization of the various species of Mycobacteria may be an aid in reclassifying these organisms. The scanning electron microscope studies so far have shown one organism to contain a flagella-like structure. The study will continue until representative examples of most of the major groups of Mycobacteria are examined.

(4) (With Dr. Esposito) The surface changes induced by an Rh antibody in Rh positive cell was studied. The Rh positive red blood cell membranes in this study did not show any changes when exposed to Rh antibody. Since Dr. Esposito is leaving DBS, further work on this project is not planned.

Significance to Bio-Medical Research and the Program of the Division: The scanning electron microscope is a comparatively recent development and its use in bio-medical research has not been exploited. The unique feature of this instrument is its depth of focus which allows cells and organisms to be studied in three dimensions giving a view of surface structure at high magnification unresolvable with any other instrument. These features should help the Division's research and safety testing programs.

Proposed Course of Project: This program will continue with emphasis on biologicals and cell surface interactions.

Honors and Awards: None

Publications: None

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Annual Report of the
Laboratory of Virology and Rickettsiology
Division of Biologics Standards

July 1, 1970 through June 30, 1971

A. ADMINISTRATIVE ASPECTS

Introduction.

During this fiscal year, continued emphasis was placed on the development of a program which would coordinate straightforward control with control-related research responsibilities. Progress has been made in phasing out old programs efficiently and developing new meaningful ones. However, the over-all operations of the Laboratory have been adversely affected by two events: Firstly, the dragging out of a poorly conducted EEO investigation caused considerable disruption of the operations of the Laboratory. Although the initial complaint was filed on 10 March 1970, as of the date of this report, the problem remains unresolved. Secondly, an amateurishly conducted formal review of the grievances of one employee was similarly dragged out resulting in additional disruption of the operations of the Laboratory.

Mission Statement. The Laboratory encompasses a broad program of research concerning the prevention and treatment of viral and rickettsial infections in man. Specific emphasis is placed upon problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. Constant liaison with the Division's other Laboratories achieves a balance in the harmonious execution of the DBS control and research responsibilities.

Personnel Changes. The full-time employee situation remained quite stable. Only two individuals (GS-5 and GS-3) were hired into positions made vacant because of death and resignation respectively.

Organizational Changes. The Laboratory of Virology and Rickettsiology (LVR) is now composed of an Office of the Chief, the Virology and Rickettsiology Section, and the Cell Biology Section. The organizational components of LVR have remained unchanged.

The Virology and Rickettsiology Section is responsible for conducting basic and applied research concerning the prevention and treatment of viral and rickettsial infections in man, with specific emphasis on problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. This Section is composed of the following 7 units: Immunoserology, Avian Leukosis, Immunofluorescence, Respirovirus, Arbovirus, Rickettsial, and Viral Pathogenesis. Nicola M. Tauraso, M.D., is Chief of this Section.

The Cell Biology Section is responsible for maintaining a facility to provide cell cultures to DBS investigators and for conducting an ongoing research program concerned with problems which might arise from the use of cell cultures for preparing virus and rickettsial vaccines. This Section is composed of the Cell Production and Cell Research Units. Henry C. Orr, Ph.D., is Chief of this Section.

B. RESEARCH ACTIVITIES

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiolcgically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

Our Laboratory was unable to confirm previously published data which showed that the A2/Aichi/2/68/X-31 so-called recombinant influenza virus strain had a growth potential far superior to the A2/Aichi/2/68 alleged parental strain. In contrast, we were able to demonstrate that, under optimal conditions, the so-called recombinant X-31 strains were able to grow only 1.5 times greater than the A2/Aichi/2/68 alleged parental strain. In fact, our results have been confirmed by all of the U. S. licensed influenza vaccine manufacturers and another independent investigator. The significance of our findings is that, at present, there is no data to support the contention that recombinant influenza virus strains can solve vaccine production problems which arise when a new A2 strain emerges in nature.

Research towards evaluating diluents and automatic diluting and pipetting machines in influenza serology led towards the development of a reliable and reproducible semiautomatic method of performing influenza serology.

We were also able to develop a quantitative radial immunodiffusion test to detect soluble residual host protein in chicken embryo-derived vaccines. This test will be employed on all influenza vaccine lots submitted for release in the hope that we will make progress in eliminating residual host protein in inactivated influenza vaccines.

Volunteer studies were performed to make comparative studies on monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. The incidence of reactions was lowest among volunteers receiving the more highly purified vaccines, such as zonal purified, ether-extracted hemagglutinin and filter-purified Sharples vaccines. Interestingly, however, there was no apparent relationship between reactogenicity and measureable avian antigen and protein nitrogen.

Surprising as it may seem, even after influenza vaccine has been in continuous use for almost 25 years, its efficacy is much in doubt. It is our feeling that this doubt has arisen from the confusion of a multitude of contradictory reports in the literature. A critical review of this literature has revealed that most investigators have not appreciated the fact that until most recently we lacked reliable methods for quantitating the antigen content of vaccines. We strongly feel that clinical evaluation studies must be supported by a critical laboratory analysis of antigen content, and a lack of knowledge of the latter precludes any meaningful conclusion of efficacy studies in man. Our influenza vaccine program was initiated with the development of a reliable and reproducible method of quantitating vaccine antigen. With this solid foundation we are progressing to volunteer studies. We feel that our methodical approach will allow us to make some definitive conclusions on influenza vaccine efficacy.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

Studies of the antigenicity and safety of the avian leukosis virus--(ALV)-free yellow fever (YF) vaccine primary seed have been completed. All of the original objectives of the YF project were realized. Candidate ALV-free primary and secondary YF vaccine seeds were developed by ridding the 17D virus vaccine seed of its ALV contaminant. The passage level of the candidate ALV-free primary seed (lot no. 17D-51) is the same as the present primary vaccine seed (lot no. AB-237) which has been employed for vaccine production for the past 25 years. The safety and antigenicity of the ALV-free YF virus strain in monkeys and man have been established. A recommendation has been made to adopt the use of the new ALV-free YF primary vaccine seed.

Progress has been made towards the development of a cell culture derived YF vaccine. Adequate virus titers were achieved in virus-infected primary chicken embryo cell cultures. Neurovirulence tests comparing the cell culture derived virus and the conventional chicken embryo derived vaccine showed these to be indistinguishable. Plans include antigenicity studies in monkeys and a clinical evaluation in man.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Recently, we have been able to use the fluorescent antibody (FAB) test to detect the virus causing Marek's disease, a contagious neuroproliferative disorder which is prevalent in chicken flocks in this country. From what is already known about Marek's virus, it is highly unlikely that this virus could survive the commonly employed procedures for preparing live virus vaccines. We will soon be able to examine finished vaccine and remove the speculation concerning the potential contamination of egg derived vaccines by Marek's virus.

The Avian Leukosis Unit of this Laboratory has been responsible for monitoring the NIH ALV-free chicken flock to insure that the flock remain free from these viruses. From our collaborative studies with a USDA geneticist we recently discovered that the NIH flock was not homogeneous genetically and that, by and large, the flock was resistant to avian leukosis subgroup B viruses (i.e., C/B). We have been able to identify and segregate some chickens with respect to their genetic susceptibility. A system of cross-breeding has been established and the embryos of trap-nested eggs are currently being screened to determine their genetic status. It is our hope that, eventually, we will be able to establish a flock which will be susceptible to viruses of all subgroups (i.e., a C/O flock). This will enable us to perform more easily our control responsibility to insure that vaccines produced in chicken embryo derived tissues are free from contaminating avian leukosis viruses. The maintenance of an avian leukosis virus genetically susceptible flock is a time-consuming, lack-lustre chore which is not fully realized by egg users, but which is nevertheless singularly necessary for certification of a "gold-bar" flock.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed towards developing a candidate lot of inactivated Typhus Vaccine for use as a reference antigen. Lots of commercially produced Typhus vaccine believed suitable for a reference vaccine have been obtained and tested by the Laboratory of Control Activities (LCA), DBS and by the manufacturer. The four candidate lots of vaccine have passed the safety, purity and potency tests ordinarily required for licensing of Typhus Vaccine. Clinical trials have been initiated to evaluate the reactogenicity and antigenicity of these preparations in volunteers.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of our Laboratory became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains of most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB, DRS (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July 1964).

We have since shown that four subsequent epizootics (namely, at the National Center for Primate Biology, Davis, California in September 1967, and at Sussex, Great Britain in December 1966, January 1968 and again in February 1969) were caused by the same virus. The significance of knowing more about potential hazardous diseases in monkeys is obvious. In addition, simian hemorrhagic fever provides a natural and safe model for the study of the pathophysiology of hemorrhagic fever. It is hoped that our studies might lead to a better understanding of hemorrhagic fever in man, and eventually might result in development of successful therapy for a disease with such high mortality rates.

Immunobiological Studies. Of major concern in studies of delayed hypersensitivity has been development of an in vitro test, particularly for man, and the discovery of the factor or factors that confer a passive hypersensitivity state. Previous studies using human RNA species in vitro have shown the transfer of an immunological message to previously nonsesnitive human lymphoid cells. Defining the mechanism of activity is paramount for evaluating RNA as a biologic product.

Studies have included: (1) isolation, purification and immunological characterization of cell antigens; and (2) an attempt to define the immunologically active molecule in a situation where RNA extracts are able to transfer antigen recognition to host lymphoid cells with which the RNA extracts incubate. These investigations show promise in such areas as: (1) identification of culture variability and antigenicity; (2) control and standardization of transplantation immunology; (3) selectivity and potency evaluation of antilymphocyte globulin; (4) tumor cell identification and standardization; and (5) a better understanding of autoimmune disorders.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Inconsistencies or failures in isolating causative agent in acute and chronic diseases of the central nervous system (CNS) constitute a major handicap in tracing the origin of the disease and relating it to certain pathogenetic events. Studies were performed to determine optimal procedures for isolation of viral agents from the CNS. Application of new isolation techniques led to rapid isolation of measles virus from monkey brains in acute model infections.

Additional studies were performed evaluating circumstances under which measles virus displays neurotropic properties and is capable of establishing an acute or chronic progressive disease of the CNS. Individual measles viruses displayed marked differences in their cultural and physico-chemical properties. Neurotropism of a strain was best related to reduced heat stability, resistance to freeze-thaw damage and susceptibility to inhibitors from brain tissue.

Knowledge of the biological properties of the neurotropic strains as well as the character of the host reaction are important for a better understanding of potential preventive or curative measures.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) developing new and improved techniques and methods for large-scale production of cell cultures; (2) developing additional cell culture systems as potential substrates for vaccine purposes; (3) studying selected biochemical parameters of cells in culture as may be related to viral susceptibility; and (4) studying factors which may influence somatic variation and modulation in cell culture systems.

As a result of some of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

C. SERVICE AND CONTROL ACTIVITIES

Cell Cultures. The Cell Biology Section continues to spend most of its effort on the purely service function of providing the entire Division with quality cell cultures for research and control uses. Much effort has been made to improve the Section's service functions and to insure adequate cell production. This service results in a considerable saving to the Division and ultimately the taxpayer. All of the DBS continuous cell lines have been replaced with certified, Mycoplasma-free cells and the cell repository is operating at an efficiency which should allow almost immediate return to full scale production in the event of accident or contamination.

Production records of primary and serial cell cultures are summarized in Tables 1 and 2. In addition, 3,480 liters of media were prepared for use in the Cell Production Unit of the Section.

Maintenance of the Cell Biology Section presents a significant monetary saving to our Division. During the 1970 calendar year, if we were to purchase the materials produced by the Cell Biology Section, it would have cost approximately the following: for primary cell cultures (Table 1), $125,899.00; for continuous cell lines, $904,125.00; for a grand total of $1,030,024.00. Maintenance of the Cell Biology Section during this same period cost the following: for salaries, $95,875.00; for materials, $20,000.00; for a grand total of $115,875.00. The minor cost of some ancillary items and services (such as bottles and bottle washing), which would add a bit to the over-all cost of producing cell cultures, has not been included in this latter total figure. Generally, by maintaining the Cell Biology Section the Division was able to realize an over-all saving of approximately $914,149.00 The fact that the quality of cells provided by the Cell Biology Section is consistently far superior to what we have been able to obtain commercially adds to the value of the Section not readily appreciated by looking at the dollar savings only.

Avian Leukosis Testing. The Avian Leukosis and Immunofluorescence Units are responsible for testing vaccines for potential contamination with viruses of the avian leukosis complex. In addition they routinely check egg derived vaccines for the presence of other avian contaminants (e.g., reticuloendotheliosis (RE) virus).

During this reporting period a total of 263 RIF (resistance inducing factor) and FAB (fluorescent antibody) tests were performed, of which 5 were on live measles vaccine, 1 on live mumps vaccine, 2 on live rubella vaccine, 12 on live Q fever vaccines, 4 on epidemic typhus vaccines.

Our Laboratory is also responsible for monitoring the NIH RIF-free chicken flock. There were 239 tests performed on eggs from SPAFAS and the NIH flocks, egg yolks, chick embryo cell cultures, and tests to evaluate drugs for antiviral activity.

Influenza Vaccines. Table 3 summarizes the potency tests performed on influenza vaccine lots submitted during this past year. In addition, in collaboration with the Division's Laboratory of Control Activities, one reference vaccine (the CCA 71C) was prepared, tested and distributed to the manufacturers.

D. PUBLICATIONS

During this fiscal year, 19 articles were published from our Laboratory. Table 4 lists the senior professional staff of LVR and the numbers of articles which they have either authored or co-authored.

E. MISCELLANEOUS

One senior professional staff member (J. Anthony Morris, Ph.D.) has failed (1) to submit annual reports during fiscal years 1969-70 and 1970-71; and (2) to report to his immediate supervisor since approximately January 1970. Attempts to take definitive action to solve this problem have been unsuccessful.

ADDENDUM

On the DBS deadline date for this Annual Report (3 May 1971), Director, DBS, submitted to this Laboratory an Annual Report written by the investigator named in the previous paragraph and typed in final. Since this latter investigator has not reported to his supervisor since January 1970, it is difficult to anticipate his erratic and unpredictable actions. The Annual Report written by Dr. J. Anthony Morris is reluctantly being included as part of this Laboratory's report because: (1) the report represents an extremely biased review of the literature; (2) the report contains untruths which should have been corrected; (3) the report also contains unfounded charges and insinuations which discredit the Division; and (4) this Laboratory was not given the opportunity to comment on this annual report while it was in draft form. It is inconceivable that the type of abuse which this annual report represents cannot be checked.

Table 1
Report for Calendar Year 1970
NUMBER OF PRIMARY CELL CULTURES ISSUED

Type of Culture	Suspension	Tubes	Bottles			
			2 oz.	MDB*	32 oz.	Other
Kidney						
Monkey, rhesus	930 ml	8,776	9,103	49	56	-
Monkey, cercopithecus	5,730 ml	81,292	4,978	214	9	-
Rabbit	18,405 ml	36,139	5,165	131	169	-
Embryo						
Chick	-	225	315	-	43	-
Mouse	-	3,812	5,400	-	-	-
TOTAL	25,065	130,244	24,961	394	277	0

MDB* = Milk dilution bottle

Table 2

Report for Calendar Year 1970

NUMBER OF SERIAL CELL CULTURES PREPARED FOR ISSUE							STOCK BOTTLES MAINTAINED	
			Bottles					
Type of Culture	Suspension	Tubes	2 oz.	MDB	32 oz.	Roller*	32 oz.	Roller
Human								
WI-38	-	7,530	2,609	49	16	23	70	330
HEp-2	-	1,724	192	130	129	54	135	71
HeLa S-3	-	1,988	398	-	-	-	49	39
FL-Amn	-	-	120	-	4	-	40	-
Animal								
Ma-104	900	54,952	39,083	-	114	-	34	871
RK-13	-	11,310	225	6	1	-	76	244
BS-C-1	750	11,798	1,040	327	16	-	80	226
L	-	300	270	-	2	-	20	35
BHK-21	-	800	460	11	36	-	224	84
Vero	8,340	21,806	10,942	-	85	-	11	254
MA-104 SHV	900	15,424	711	-	2	-	250	91
MA-104 C-1	-	8,500	675	-	2	-	43	78
Rabbit Embryo	-	690	-	-	-	-	19	-
Rat Embryo	-	-	12	-	-	-	-	42
MA-111	-	715	-	-	-	-	86	-
MA-134	-	4,000	-	-	-	-	31	42
Totals:	10,890	141,537	56,737	523	407	77	1,168	2,416

*Each roller bottle is the equivalent of 6 32-oz. bottles

Table 3
Report for Calendar Year 1970
SUMMARY OF POTENCY TESTS ON INFLUENZA VACCINES

Manufacturer No.	Vaccine Type[a]	Total Lots Submitted (No.)	Lots Tested (No.)	Lots Released (No.)	Lots Rejected or Withdrawn by Mfgr.
1	CB	4	4	4	0
	Mil.	1	1	1	0
2	CB	2	2	2	0
3	CB	11	11	11	
4	CB	14	14	14	0
	Mil.	9	9	9	0
5	CB	20	14	20	0
6	CB	11	11	10	1
Total	CB	62	56	61	1
M	Mil.	10	10	10	0

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[a] CB = Civilian Bivalent; Mil. = Military polyvalent vaccines.

Table 4. Senior Professional Staff and Summary of Publications Realized During Fiscal Year 1970-71.

Investigator	Number of Publications	
	Senior Authorship	Co-authorship
Paul Albrecht, M.D.	3	2
Calvin G. Aulisio, Ph.D.	-	1
William G. Jahnes	-	1
Richard A. Mason, D.V.M., Ph.D.	1	-
J. Anthony Morris, Ph.D.[a]	Information not available	
Martin G. Myers, M.D.	-	3
Thomas C. O'Brien, Ph.D.	5	1
Henry C. Orr, Ph.D.	-	-
Alan V. Richman, M.D.	1	-
Nicola M. Tauraso, M.D.	7	10
Daniel E. Thor, M.D., Ph.D.	1	3
Chen P. Li, M.D.	1	-

[a] Investigator failed to submit annual reports during fiscal years 1969-70 and 1970-71.

Serial No. 57
1. Virology & Rickettsiology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Office of the Chief

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives: Administration, planning and coordination of research and control activities of the Laboratory's sections.

The project furnishes supervisory and administrative services to all research projects and biologics control activities in the laboratory as follows: (1) over-all planning of intramural research, collaborative (contract) and control functions and their coordination among and within the several Sections of the Laboratory; (2) supervision over personnel, maintenance, travel and correspondence; (3) supervision over requisitioning and maintenance of equipment and supplies; (4) reviewing, editing and revising scientific and technical reports and manuscripts; (5) preparation of administrative reports, exhibits and other publication materials; (6) participating with other Laboratory Chiefs in staff functions for Office of the Director, including staff committees which ensure smooth operation of the Division in its many types of activity; (7) official liaison and communications with the manufacturers of viral vaccines and other biologics; (8) consultation services to individuals, academic institutions and government agencies, including liaison activities with other branches of the service, foreign governments and international agencies; (9) maintenance of reference library and reprint files.

Serial No. 57

Honors and Awards:

1. Invited participant, Seminar on Microbiological Aspects of Colony Management, American Association of Laboratory Animal Science, 21st Annual Session, Chicago, Illinois, 2-6 November 1970 (Review of Recent Epizootics in Primate Colonies and Their Relation to Man).
2. Invited participant, Smiian Virus Workshop, sponsored by the Animal Resources Branch, Division of Research Resources, NIH and the Southwest Foundation for Research and Education (SFRE), SFRE, San Antonio, Texas, 26-27 April 1971.
3. Associate Member, Commission on Influenza, Armed Forces Epidemiological Board, June 1969 - June 1971.

Publications:

1. Tauraso, N. M.: Review of recent epizootics in subhuman primate colonies and their relation to man. Lab. Animal Care. In Press.

Serial No. 139
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on autoimmune reactions related to viral and adjuvant sensitization

Previous Serial No.: Same

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: Nicola M. Tauraso, M.D.
Thomas C. O'Brien, Ph.D.
Ruth L. Kirschstein, M.D.
Alan Shabo, M.D.

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 1.3
Professional: 0.8
Other: 0.5

Project Description:

Objectives: Evaluate circumstances under which measles virus displays neurotropic properties and is capable of establishing an acute or chronic progressive disease of the central nervous system (CNS).

Methods Employed: Tests for humoral and cell-mediated immunity were performed on monkeys developing natural measles infection or on monkeys stimulated with antigen in adjuvants. Measles-free monkeys and rats of the Lewis strain were injected intracerebrally with a hamster-adapted neurotropic measles virus (strain HNT) and the resulting infection examined by virological techniques, fluorescent antibody staining and histology. In some animals delayed hypersensitivity to measles antigen or suppression of the immune response were induced.

Major Findings: A benign encephalitis developed in both rhesus monkeys and rats inoculated with the neurotropic measles virus strain. Virus was localized in nerve and glial cells. Inflammatory lesions spread over large parts of the CNS. In immunosuppressed animals the disease took a lethal course.

In rats encephalitis could be prevented or, when established, could be eliminated by stimulation of humoral and cell-mediated immune response. Studies in rats and monkeys indicated that the organism as a rule fails to react to measles infection with a cell-mediated immune response. The inability to mount delayed hypersensitivity may be the main reason for the chronic, relentless course of measles encephalitis.

Significance to Bio-Medical Research and Program of DBS: This is the first instance of an experimental measles encephalitis in a primate. The infection provides a relevant model to a) compare neurotropic properties of different measles virus strains, b) evaluate possible therapeutic measures in human measles encephalitis (transfer of delayed hypersensitivity, interferon induction), and c) examine the hypothesis of interaction of measles virus with other viral agents in the development of chronic measles encephalitis (SSPE).

Proposed Course of the Project: Further experiments will attempt to produce a more protracted course of measles encephalitis in monkeys and examine the effect of therapeutic measures on the infection.

Honors and Awards: None

Publications: None

Serial No. 173
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Improved techniques for isolation and study of viral agents affecting the nervous system.

Previous Serial Number: Same

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: N. M. Tauraso, M.D.
T. C. O'Brien, Ph.D.
A. L. Shabo, M.D.
Ruth L. Kirschstein, M.D.

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 1.7
Professional: 1.2
Others: 0.5

Project Description:

Objectives: Measles virus has been implicated in chronic forms of encephalitis and encephalopathy in man. Measles virus with restricted growth properties was transferred into cell cultures and rendered cell-free by special procedures. A considerable proportion of recently observed patients with chronic measles encephalitis (subacute sclerosing panencephalitis - SSPE) has been involved in the mass measles vaccination program. However, a causal relationship of SSPE to measles vaccination has not been established.

Methods Employed: Studies were performed to A) determine physical and biological properties of measles virus strains with different pathogenic properties for man, and B) examine common laboratory animals for their susceptibility to measles neuropathogenicity.

Major Findings:

1. Significant differences were found among measles virus strains concerning plaque size in cell cultures, interferon production, heat stability and susceptibility to inhibitors. The most significant quality distinguishing the live vaccine strains from non-vaccinal strains was the capacity of the former to grow to high titers in chick embryo cell (CEC) cultures. This property was retained after adaptation to and 10 consecutive passages in hamster brain. SSPE-derived measles virus strains, including one strain recovered from a patient one year after measles vaccination, lacked significant proliferation capacity in CEC cultures. One SSPE isolate, claimed by the original authors to multiply in CEC cultures to high titers, lacked this property in our laboratory

after being separated from mycoplasma contamination.

2. Suckling hamsters proved considerably more susceptible to intracerebral inoculation of measles virus than suckling mice. Neuropathic properties of different measles virus strains ranged from discrete morphological lesions to severe lethal encephalitis. Original measles vaccine strains had the lowest activity in hamsters. Contrary to expectation, viruses derived from brains of patients with SSPE were not lethal for hamsters. The neuropathogenicity of measles virus strains for hamsters could be altered through passage in different cell substrates. Further experiments are necessary to see whether a correlation exists between the neuropathogenicity of measles virus for hamsters and for primates.

3. Eighteen rhesus monkeys were inoculated intracerebrally with the following three Vero cell-passaged viruses: a) A low-passage "wild" strain; b) the attenuated Schwarz strain; and c) the hamster neurotropic (HNT) strains of measles virus. The hamster-neurotropic strain produced clinical and histological encephalitis. The "wild" and attenuated strains, although possessing some neuropathogenicity for hamsters, were not encephalogenic for rhesus monkeys. Immunosuppression of the animals which considerably aggravated the infection with the hamster-neurotropic strain still showed the "wild" and attenuated strains to be devoid of encephalitogenic properties for rhesus monkeys.

4. During our studies on the isolation of neurotropic measles virus from brain tissue and the course of measles virus encephalitis in rhesus monkeys, optimal conditions were established for the dispersion and cultivation in cell culture of rhesus brain cells. Several latent foamy agents were recovered in brain cultures processed in the above-mentioned way. Cross-neutralization tests with nine foamy virus isolates revealed two distinct foamy virus serotypes (types 1 and 2).

Significance to Bio-Medical Research and Program of DBS: The increased interest in CNS infections caused by measles or measles-like viruses makes a search for stable markers associated with certain pathogenic properties of measles virus desirable. Although several studies have shown the isolation of foamy virus type 1 from primary rhesus monkey kidney cell cultures, this is the first report of the isolation of foamy virus type 1 from primary rhesus brain cultures and the first report of the isolation of foamy virus type 2 from rhesus monkeys. This indicates that these latent agents are probably equally prevalent in the tissues of rhesus monkeys, the only deterrent to their detection being the technique of isolation of the various primary tissues.

Proposed Course of the Project: Studies on the properties of different measles virus strains will continue at the present rate.

Honors and Awards:

1. Invited participant (Dr. P. Albrecht) to the VIth International Congress of Neuropathology, Paris, Agust 31 - September 4, 1970 (Immune Status of the Organism During Experimental Scrapie Infection).

Publications:

1. Schumacher, H. P. and Albrecht, P.: Optimal conditions for isolation of a neurotropic measles virus from brain tissue. Proc. Soc. Exp. Biol. Med. 134: 396-402, 1970.

2. Albrecht, P. and Schumacher, H. P.: Viability and infectivity of measles virus-infected nervous tissue frozen in cryoprotective medium. Appl. Microbiol. 20: 160-163, 1970.

3. Albrecht, P. and Schumacher, H. P.: Neurotropic properties of measles virus in hamsters and mice. J. Inf. Dis. In press.

4. Albrecht, P.: Immune status of the organism during experimental scrapie infection. Proc. VIth Internat. Congr. Neuropathology, Masson & Co., Paris, 1970.

5. O'Brien, T. C., Albrecht, P., Schumacher, H. P., and Tauraso, N. M.: Isolation of foamy virus types 1 and 2 from primary rhesus monkey brain cultures. Proc. Soc. Exp. Biol. and Med. In press.

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Serial No. 75
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on arthropod-borne viruses (arboviruses)

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: George M. Bernier, M.D.

Cooperating Units: Department of Hematology, Walter Reed Army Institute of Research, Department of the Army, Washington, D. C.

Man Years:
Total: 0.74
Professional: 0.49
Other: 0.25

Project Description:

Objectives: (1) To maintain a laboratory facility with competency in the techniques of animal work, cell culture and serology for the study of arboviruses; (2) to maintain a reference collection of arboviruses, including those of importance as vaccine candidates; and (3) to conduct studies on select arboviruses, especially those of importance as potential vaccine candidates.

Major Findings: The arbovirus facility now has the professional and technical competency capable of performing studies on the arboviruses, utilizing the techniques of animal inoculation, cell culture and serology.

The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains for most of the major and minor arbovirus groups are available and are being utilized for routine and special studies. During this year studies were continued with emphasis on simian hemorrhagic fever, American hemorrhagic fever and yellow fever vaccine viruses (reported separately under project Nos. 118 and 121 respectively).

Serial No. 75

The effects of various commonly used procedures for removing non-specific viral inhibitors upon serum proteins and immunoglobulins were evaluated. Several types of effects were observed. Treatment with ether, chicken red blood cells, receptor destroying enzyme (RDE), and RDE plus heat (65°C/30 minutes) resulted in a generalized increase in the immunoglobulin concentration. Conversely, treatment with RDE plus mercaptoethanol and with periodate produced a generalized decrease in immunoglobulin concentration.

Significance to Bio-Medical Research and the Program of DBS: Many of the continental states, as well as Hawaii, Alaska and Puerto Rico, possess the ecological prerequisites of potential vectors and reservoirs necessary for initiation of arbovirus epidemics. Vaccines against arboviruses of importance to the world--the U. S. personnel and travelers abroad--are being developed. Some may soon come within the purview of the Division of Biologics Standards. Further knowledge of the biochemical and biophysical properties is needed in order to appreciate more the unique features of arboviruses and before these viruses can be handled as biologic products.

Proposed Course of the Project: Research on selected arboviruses will continue.

Honors ans Awards: None

Publications:

1. Tauraso, N. M., Pedreira, F. A. and Bernier, G. M.: Effect of various methods for removing nonspecific inhibitors to virus virus hemagglutination upon serum proteins and immunoglobulins. *Archiv. ges. Virusforsch.* In Press.

Serial No. 118
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on hemorrhagic fever viruses

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Martin G. Myers, M.D.

Other Investigators: Calvin G. Aulisio, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.71
Professional: .88
Other: .83

Project Description:

Objectives: A. (1) To maintain a facility for working with the important viruses causing hemorrhagic fever in the western hemisphere with emphasis on the immunological vaccine aspects; (2) to maintain practical cell culture and animal host systems and serological tools for working with this group of viruses; (3) to study the immunological relationships of these viruses using in vitro serological tests and in vivo animal protection tests.

B. (1) To study the pathophysiology of hemorrhagic fever using monkeys infected with simian hemorrhagic fever (SHF) virus as a model; (2) to attempt to isolate a virus from specimens obtained from monkeys which died of hemorrhagic fever at the National Center for Primate Biology, University of California at Davis in September 1967 and at a non-human primate colony in Sussex, Great Britain in 3 successive years from 1966-69; and (3) to determine the relationship of these outbreaks with the hemorrhagic fever which occurred at the Primate Quarantine Unit, NIH.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings:

A. Species Susceptibility of SHF:

Thus far, only monkeys of the Macaca species have acquired the natural disease. Primates which have been naturally exposed in one or more of the epizootics but have not contracted the disease include the squirrel monkey (*Saimiri sciurea*), chimpanzees (*Pan satyrus*), baboons (*Papio papio*), and man.

We are currently investigating what non-human primate species are susceptible to SHF; thus far, illness has been reproduced in patas and African green monkeys which nevertheless survived. In addition, two gelada baboons, seroconverted after experimental exposure to SHF.

In order to determine the natural source of the virus, a serologic survey was performed. The results were somewhat surprising in that extensive surveillance of numerous primate sera of widely diverse species (man, gorilla, chimpanzee, orangutan, gibbon, baboon, African green, rhesus, cynomolgus, talapoin, stump-tailed macaque, and marmosets) for CF antibody to SHF virus was consistently negative. Several patas monkeys have been found to have pre-existing CF antibody to SHF virus.

B. Virology of the SHF epizootics:

Difficulties encountered in the initial isolations of SHF were a manifestation of the marked pathogenic specificity of the virus. Thus far, only monkeys of the *Macaca* species appear to be susceptible to natural infection. Many unsuccessful attempts have been made to isolate SHF virus in golden Syrian hamsters, guinea pigs, white mice, white rats, rabbits, and embryonated chicken eggs. Initial attempts to isolate SHF virus in a variety of cell cultures also failed,until the MA-104 embryonic rhesus monkey kidney cell line was used. After the virus had been isolated and passaged 8 times in MA-104 cells, the virus still would not infect any of the animals mentioned above nor would it replicate in many primary or continuous cell cultures. The MA-104 cell-culture-adapted virus could replicate in only one other cell line, the BS-C-1 African green monkey continuous cell line.

Current studies have revealed marked differences in the susceptibility of different MA-104 tissue culture cell lines to the different strains of SHF. Subsequently, an agent has been isolated from the Sussex/69 epizootic which is indistinguishable from those previously isolated by FAB and CF.

Continuing attempts to isolate an agent from the Davis/67 epizootic has been unsuccessful and may be related to the unusual species and cellular specificity of SHF. Comparison of the agents from the four epizootics by CF, using infectious sera or tissue culture derived antigens, have shown the agents to be serologically indintinguishable.

Significance to Bio-Medical Research and the Program of DBS: A. Concerning the studies on the American hemorrhagic fever viruses, there is no doubt that viral hemorrhagic fevers have become a major public health concern in the Americas. So far attempts to provide a safe and adequate vaccine against the Argentinian disease have been unsuccessful. During the most recent epidemics of Bolivian hemorrhagic fever, much consideration was given to the possibility of immunization as an eventual control measure. It would be in keeping with the mission of this Division to develop and maintain the competency able to cope with the problems of evaluating the potential vaccine candidates.

B. Since primates are needed by the Division in carrying out the required safety tests on some vaccines, it is only logical that the arbovirus facility of LVR maintain a competency able to cope with certain aspects of endogenous viral diseases of monkeys.

During the years of 1964 to 1969 there have been six known outbreaks of simian hemorrhagic fever. The significance of knowing more about potential hazardous diseases of monkeys is obvious.

Proposed Course of the Project: Studies on the American hemorrhagic fever and simian hemorrhagic fever viruses will be continued.

Honors and Awards: None

Publications:

1. Shelokov, A., Tauraso, N. M., Allen, A. M., and Espana, C.D.: Epizootic, clinical and pathological aspects of simian hemorrhagic fever. In Marburg Virus Disease, Springer-Verlag, Berlin-West, 1970, pp. 153-157.

2. Tauraso, N. M., Aulisio, C. G., Espana, C. D., Wood, O. L., and Liebhaber, H.: Simian hemorrhagic fever virus. In Marburg Virus Disease, Springer-Verlag, Berlin-West, 1970, pp. 158-163.

3. Wood, O. L., Tauraso, N. M., and Liebhaber, H.: Electron microscopic study of tissue cultures infected with simian hemorrhagic fever virus. J. Gen. Virol., 7: 129-136, 1970.

4. Tauraso, N. M., Myers, M., McCarthy, K., and Tribe, G. W.: Simian hemorrhagic fever. In Infections and Immunosuppression in Sub-Human Primates, H. Balner and W. I. B. Beveridge (Ed.), Munksgaard, Copenhagen, Denmark, 1970, pp. 101-109.

5. Tauraso, N. M., Kalter, S. S., Ratner, J. J., and Heberling, R. J.: Simian hemorrhagic fever. In Proceedings of the Second Conference on Experimental Surgery in Primates, New York, N.Y., 7-12 September 1969.

Serial No. 121
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Yellow Fever Vaccine (YFV)

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Richard A. Mason, D.V.M.

Other Investigators: William G. Jahnes
Ruth L. Kirschstein, M.D.
Freddie Mitchell, M.S.
Alan V. Richman, M.D.
Edward B. Seligmann, Jr., Ph.D.

Cooperating Units: Laboratory of Control Activities, DBS
Laboratory of Pathology, DBS

Man Years:
Total: 3.09
Professional: 1.92
Other: 1.17

Project Description:

Objectives: (1) To purify the current Yellow Fever Vaccine by removing the contaminating avian leukosis virus (ALV); (2) to evaluate the neurovirulence of the new vaccine candidate in monkeys; (3) to study the immunogenicity of the purified vaccine candidate in monkeys and man; (4) to develop a cell culture derived vaccine; and (5) to determine the relationship between vaccine dose and immunogenicity.

Methods Employed: Utilizing the broad resources available to the Arbovirus, Immunoserology, and Avian Leukosis Units, LVR, an attempt is being made to investigate the various aspects of this project by common laboratory techniques.

Major Findings: The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division.

A. Laboratory Investigations:

1. Studies have been underway to develop a YFV in cell cultures. Adequate virus titers were achieved in virus-infected primary chicken embryo cell cultures growing at 32°C, and harvesting at 3 days appeared to be ideal conditions. Neurovirulence tests comparing the cell culture-derived virus and the conventional chicken embryo derived vaccine showed these to be indistinguishable. A comparison of cell culture-derived virus grown at several temperatures is currently being performed.

2. Studies are being performed to select the optimal combination of media and stabilizers to permit lyophilization and storage in convenient rubber-sealed vials. This will obviate the use of glass-sealed ampoules which have been both inconvenient and cumbersome.

3. Studies on the antigenicity of the ALV-free YFV in rhesus monkeys have been completed. The results of serological tests (i.e., neutralization and hemagglutination-inhibition) revealed that the ALV-free and ALV-contaminated lots were essentially comparable.

4. A total of 456 rhesus monkeys were inoculated with varying dilutions of YFV. Table I shows the immunization schedule, numbers of monkeys and vaccine dosages employed. Plaque neutralization (PN) tests were performed on 137 triplicate serum samples, and hemagglutination-inhibition (HI) tests employing 7 arbovirus antigens, including the 17D and FN strains of yellow fever viruses, were performed on 128 triplicate serum samples. Table II shows the PN and HI antibody responses. Essentially, the responses were similar in terms of group mean titers and percent conversions in monkeys receiving 10-fold to one-tenth the usual human vaccine dose (Groups 1 to 3). Average PN titers appeared as high in monkeys given one-hundredth the human dose (Group 4); however, the percentage of conversions was lower (67% to 79%) than the higher dosages.

Major differences between PN antibody responses at 4 and 8 weeks after vaccination have not been noted.

B. Volunteer Studies:

Reactogenicity and antigenicity studies in man. Two separate volunteer studies were performed. In the first, conventional ALV-contaminated YF vaccine was given to 89 and ALV-free vaccine was administered to 91 inmates (Lewisburg Federal Penitentiary, Lewisburg, Pennsylvania). The vaccines were indistinguishable with respect to reactogenicity and antibody response (Table IV). One year following vaccination, serum was obtained from the remaining volunteers and tested with the serum samples obtained prior to and 28 days after vaccination for the presence of HI and PN antibodies. Considering the results of the more sensitive PN test, all volunteers remaining one year after vaccination demonstrated significant levels of serum antibody (Table III).

In the second volunteer study, four different lots of conventional ALV-contaminated vaccine were given to 827 and four lots of ALV-free vaccine to 675 U.S. Army recruits (Ft. Ord, California). The total number of subjective and objective reactions was minimal, and the same in both groups, indicating a comparably low degree of reactogenicity of the 8 vaccine lots tested.

Sera from approximately 25 volunteers from each of the 8 immunization groups were examined serologically. All vaccine lots were indistinguishable with respect to HI and PN antibody response (Table III).

Significance to Bio-Medical Research and the Program of DBS: A re-evaluation of old vaccines using more up-to-date methods is one of the responsibilities of the Division. The development of a Yellow Fever Vaccine free of contaminating avian leukosis virus is obviously advantageous to humans receiving this vaccine.

Proposed Course of the Project: These studies are being continued.

Honors and Awards: None.

Publications: None.

Table I. Numbers of Monkeys and Vaccine Dosages Used in Study

Vaccine Dilution Inoculated	Experiment Number/Dosage					
	1	2	3	4	5	Subtotal/Range
Undil.	13/6.3[a]	16/5.9	19/6.6	15/5.4	15/6.4	78/5.4 - 6.6
-1	12/5.5	14/5.3	16/4.8	17/4.6	17/5.5	76/4.6 - 5.5
-2	12/4.0	14/4.8	16/3.7	17/3.5	17/3.4	76/3.4 - 4.8
-3	12/ND	14/2.5	16/2.7	17/1.5	17/2.7	76/1.5 - 2.7
-4	12/0.0	14/0.0	16/0.0	17/0.0	17/1.7	76/0.0 - 1.7
-5	12/ND	14/0.0	16/0.0	17/0.0	15/0.0	74/0.0
Subtotal	73	86	99	100	98	456/N.A.[b]

[a] Numbers of monkeys/suckling mouse intracerebral LD_{50} ($\log_{10}$) per 0.5 ml inoculum administered to each monkey.

[b] Not applicable.

Table II. Hemagglutination-Inhibition and Plaque Neutralization Antibody Responses of Monkeys Inoculated with Varying Dilutions of Yellow Fever Vaccine

		HI[b]			PNT[c]/Percent[d]		
Group	Dosage[a]	No. Tested	4 wk.	8 wk.	No. Tested	4 wk.	8 wk.
1	5.4-6.6	23	67-100	57-100	25	2.5/100	2.8/95
2	4.6-5.5	27	50-100	50- 83	23	3.0/100	2.7/100
3	3.4-4.8	21	20-100	20- 83	21	2.7/95	3.2/100
4	1.5-2.7	20	50-100	33-100	22	2.0/68	2.2/ 79
5	0.0-1.7[e]	20	0- 75	0- 50	23	0.78/25	0.82/30
6	0.0	17	0- 67	0- 67	23	0.11/ 0	0.04/ 0
Total		128			137		

[a] Range of intracerebral suckling mouse LD_{50}/0.5 ml administered to numbers of monkeys shown. Combined data of four trials.

[b] Percent of monkeys developing a 4-fold or greater rise in HI antibody titer.

[c] PNT = plaque neutralization test. Expressed as log neutralization index (LNI) for each group of monkeys shown.

[d] Percent of monkeys having an LNI $\geq$ 0.7.

[e] No virus detected in 4 weanling mouse tests; value = 0.0 in three of four suckling mouse tests.

Table III. Results of Serological Tests Performed on Volunteers Inoculated with ALV-Contaminated and -Free Yellow Fever (YF) Vaccine

Group	ALV Status of Vaccines							
	Contaminated				Free			
			PNT[b]					
	No. in Group	HI[a] %	LNI[c]	%[d]	No. in Group	HI %	LNI	%
Lewisburg Study initial survey (28 days)	89(65)[f]	62(71)[g]	2.2[e]	99	91(68)	69(78)	2.2	98
1 yr survey	22(18)	86(77)	2.9	100	17(10)	77(100)	2.7	100
(28 days)		45(45)	3.5	100		47(70)	2.9	100
(1 yr)								
Ft. Ord Study								
1A51[h] (5A21)[i]	25(22)	92(91)	3.1	100	23(17)	96(100)	3.1	100
2D13 (6B52)	24(19	91(95)	2.7	100	23(22)	100(100)	2.8	100
3D21 (7B43)	25(23)	100(100)	2.8	100	25(21)	92(91)	2.4	100
4H43 (8A53)	25(14)	92(100)	3.0	100	25(16)	96(100)	2.8	100
Ft. Ord Total	98(78)	94(96)	2.9	100	96(76)	96(97)	2.8	100

[a] Percent of individuals developing a 4-fold or greater rise in HI antibody titer.

[b] PNT = plaque neutralization index.

[c] LNI = log neutralization index.

[d] Percent of individuals having an LNI of $\geq$0.7.

[e] Average value.

[f] Number with no YF HI antibody in preinoculation serum.

[g] Percent of individuals not having YF HI antibody in preinoculation serum who developed 4-fold or greater rise in HI antibody.

[h] First column contains the group numbers of those receiving the conventional ALV-contaminated vaccines.

[i] Second column contains the group numbers in parentheses of those receiving ALV-free vaccines.

Serial No. 122
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on respiratory viruses.
I. Influenza virus vaccines

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Thomas C. O'Brien, Ph.D.
Martin G. Myers, M.D.

Other Investigators: Capt. Robert O. Peckinpaugh
Alan V. Richman, M.D.

Cooperating Units: Naval Medical Research Unit No. 4,
Great Lakes Naval Training Center,
Great Lakes, Illinois

Man Years:
Total: 8.76
Professional: 2.68
Other: 6.08

Project Description:

Objectives: (1) To maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza viruses and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform immunogenic potency tests on current influenza virusvaccines; (4) to improve serologic techniques for immunogenic potency testing of influenza virus vaccines; (5) to compare reproducibility of serologic tests for immunogenic potency of current influenza virus vaccines; and (6) to conduct immunogenic studies with influenza viruses of epidemiologic importance in the United States.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

When a comparison of the sensitivity of test methods and diluents was made for determining HA titers, the AUTO microtiter procedure and PBS/GEL diluent appeared to be the method and diluent of choice.

Evaluation of another instrument, the autopipetter, which standardizes the volume of diluent to be added in the microtechnique, suggests that the reproducibility of the AUTO microtiter procedure might be further increased.

3. To avoid the problems of allergic sensitization against egg protein or of eliciting immediate or delayed hypersensitivity in a previously sensitized host, radial immunodiffusion was used with ovalbumin as the reference protein, to quantitate residual host protein in chicken embryo-derived vaccines.

Six replications each of the following concentrations of ovalbumin (1,2,3,4,5,6,7,8,9,10,15,20,30 and 35 mcg/ml) were tested by radial immunodiffusion. Regressions were computed using the area measurement of response. It was found that the 6 replications could be fitted to a straight line separately. A Bartlett's test for homogeniety of the variances of the 6 replications was barely significant at the 5% level. A covariance of the 6 replications showed no significant difference in the slopes, nor the adjusted means, so the data of the 6 replications was pooled to form a single straight line. Influenza virus vaccines from six manufacturers (1967, 1968, and 1969 formulations); measles virus vaccine, avianized smallpox virus vaccine, yellow fever virus vaccine, and dilutions of chicken embryo homogenate were analyzed for their concentration of egg protein. All vaccines tested were undiluted. Smallpox, yellow fever, and 1967 and 1968 influenza virus vaccines produced by the Sharples process had significant quantities of residual soluble host protein. 1969 influenza virus vaccines either had no detectable residual protein or borderline (1 mcg/ml) quantities.

Quantitative radial immunodiffusion is an economical, rapid and accurate method for the detection of soluble residual host protein in chicken embryo-derived vaccines.

B. Volunteer Studies.

1. A variety of new techniques for the production of influenza vaccine are now available. Zonal ultracentrifugation has led to the development of relatively homogeneous vaccines, antigenically. Tissue culture methods have been enlisted for vaccine production making possible the further reduction of local

and systemic reactions caused by sensitivity to avian antigens incident to vaccine production in chicken eggs. However, these factors have also contributed to the current situation in which numerous variables among vaccines with respect to antigenic content and toxicity are compounded by various methods of preparation, routes of administration and multivalent composition.

In this light, it has been a matter of special interest to make comparative studies of monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. Studies to evaluate differences in the methods of vaccine preparation and routes of administration were, therefore, performed at the Great Lakes (Illinois) Naval Training Center in collaboration with the Naval Medical Research Unit No. 4. In the first study, a total of twenty-two formalin inactivated monovalent A2/Aichi/2/68 influenza vaccine lots, containing approximately 400 chicken cell agglutinating (CCA) units per dose, were administered to approximately 2800 recruits. The vaccines were prepared by seven commercial firms by four different methods and were injected by either the subcutaneous or intramuscular routes, as were the five corresponding saline placebo preparations. Essentially, vaccines were prepared by one of four methods: The conventional Sharples techniques, zonal ultracentrifugation, and ether-extracted Sharples or zonal concentrates. All vaccine lots were prepared in the chicken embryo allantoic sac except for the zonal/HA vaccines which were derived from bovine kidney tissue cultures. Matched "sister" lots of Sharples and Sharples/HA vaccines were prepared with and without aluminum phosphate adjuvant. We included two or more vaccine lots from each manufacturer in order to evaluate lot to lot, as well as manufacturer to manufacturer, variation. Detailed laboratory analyses of the vaccines employed in this study were performed to help evaluate antigenicity and reactogenicity of the vaccine preparations. Each of the resultant 47 vaccine and placebo groups was administered to approximately 60 men.

The incidence of reactions was lowest among recruits receiving the more highly purified vaccines, such as zonal purified (regardless of the presence of gelatin), ether-extracted hemagglutinin, and filter-purified Sharples vaccines. Interestingly however, there was no apparent relationship between reactogenicity and measureable avian antigen, protein nitrogen nor the amount of particulate matter introduced into the recruits. Our results show that vaccines containing $AlPO_4$ adjuvant were uniformly more toxic than both the other vaccines and their matched vaccine lots prepared without adjuvant.

Serial No. 122

Major Findings:

A. Laboratory investigations:

1. Final results are reported on initial experiments reported in 1970 on the comparison of the hemagglutinin production of influenza A2 Hong Kong variant and recombinant strains.

Seven Hong Kong variant strains were compared for their capacity to produce hemagglutinin (HA). From the results of 9 serial egg passages made at 33°C and 36°C, the A2/Aichi/2/68 strain was found suitable as the vaccine production strain for the 1968-69 monovalent influenza vaccine manufactured in the United States.

Results of initial experiments comparing the HA production of 2 Hong Kong variant recombinants and the A2/Aichi/2/68 strains revealed essentially no difference among these strains. When these experiments were repeated starting with viruses which had the same number of egg passages from isolation, it was found that the mean HA and the chicken cell agglutination (CCA) titers of the recombinant strains used in these studies were only 1.5 times greater than the A2/Aichi/2/68 strain. This did not compare with the HA producing capacity of AO/PR/8/34 which was almost 6 times the A2/Aichi/2/68 strain. These results are in contrast to results reported by others.

2. Statistical evaluation was performed on diluents and automatic diluting and pipetting machines in influenza serology.

The use of three diluents (i.e., 0.01 M phosphate-buffered saline. PBS; PBS with 0.2% gelatin, GEL; and PBS with 0.4% bovine plasma albumin, BPA) and three methods (i.e., the standard tube macro-procedure, TUBE; the manual microtechnique, MANUAL; and the semiautomatic microtechnique, AUTO) were statistically compared for their reproducibility and sensitivity in determining hemagglutinin (HA) and hemagglutination-inhibition (HI) antibody titers. In the HA test, analyses of between-cell variances of the different methods showed the AUTO microtiter procedure to be more reproducible than the standard TUBE method. The MANUAL microtiter procedure was the least reproducible. In the HI test, the TUBE method was the most reproducible. No significant difference in the reproducibility of the diluents was observed in either the HA or HI test.

When this study was initiated, most manufacturers of inactivated influenza virus vaccine in the United States were still preparing vaccines by the conventional Sharples process. Three manufacturers were preparing more purified vaccines, and several were in the process of converting from conventional to more purified processes. Presently, all but one manufacturer prepares more purified influenza vaccines. The results of our study indicate, despite marked lot to lot variations in reactogenicity, that the use of more purified vaccines results in less local and probably reduced systemic reactions.

Analyses of serological testing are currently under way.

2. Because of the controversy concerning the immunogenicity of influenza antigens given subcutaneously (SQ), intradermally (ID), or intranasally (N), a second study was undertaken to investigate serum and nasal antibody production by these three routes of immunization. The vaccine used was a zonal centrifuged preparation containing from 53 to 2139 CCA units for the SQ and N routes and 12.7 to 515 CCA units for the ID route. Reactions to the parenteral injection were minimal, even at the highest doses administered. There were no adverse reactions to the nasal immunization. Even the smallest dose given ID (12.7 CCA units) resulted in a significant seroconversion rate. Seroconversion rates were 85 to 86% with a vaccine dosage ranging from 34 - 213.9 CCA units. When the dosage was increased to 515 CCA units, 100% of the subjects converted. A similar pattern is seen with doses given by SQ except 963 CCA units were required to produce a 100% seroconversion rate. The intranasal route was less effective in stimulating serum antibody and seroconversion rates did not reach 100% with any of the doses given. Recruits with an initial antibody titer of 1:8 and immunized by either SQ or ID routes, showed a 100% seroconversion rate at all doses used. The intranasal route did not stimulate a 100% seroconversion rate until a dose of 953 CCA units was used. In those recruits who had an initial antibody of 1:8 or greater, the seroconversion rate was noticeably reduced.

Significance to Bio-Medical Research and the Program of DBS: DBS is responsible for the control and potency testing of current influenza virus vaccines. Sustained and comprehensive scientific research, as performed by the Respirovirus Unit, is essential for the improvement and development of antigenically competent influenza virus vaccines.

Proposed Course of the Project: Studies on influenza viruses and influenza vaccines will continued.

Honors and Awards: None

Publications:

1. Tauraso, N. M., and O'Brien, T. C.: Comparison of the hemagglutinin production of influenza A2 Hong Kong variant and recombinant strains. Bull. Wld. Hlth. Org., 43: 275-279, 1970.

2. O'Brien, T. C., Rastogi, S., and Tauraso, N. M.: Statistical evaluation of diluents and automatic diluting and pipetting machines in influenza serology. Appl. Microbiol., 21: 311-315, 1971.

3. O'Brien, T. C., Maloney, C. J., and Tauraso, N. M.: Quantitation of residual host protein in chicken embryo-derived vaccines by radial immunodiffusion. Appl. Microbiol.: In Press.

4. Edwards, E. A., Peckinpaugh, R. O., Rosenbaum, M. J., Mammon, R., Tochman, M., Frazier, W., Tauraso, N. M., and Myers, M. G.: Influenza vaccine: Primary and booster response to influenza vaccine by dose and route of administration. Bull. WHO. In Press.

5. O'Brien, T. C., Yahwak, R. S., and Tauraso, N. M.: Effect of freezing and lyophilization on the chicken cell agglutination (CCA) activity of inactivated influenza virus vaccines. Archiv. ges. Virusforsch., 32: 39-44, 1970.

6. Richman, A. V., and Tauraso, N. M.: Attempts to demonstrate hemagglutination and hemadsorption by respiratory syncytial virus. Appl. Microbiol. In Press.

Serial No. 209
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of the relative immunogenicity of two live virus vaccines given at various intervals

Previous Serial Number: None

Principal Investigators: Nicola M. Tauraso, M.D.
Thomas C. O'Brien, Ph.D.

Other Investigators: Martin G. Myers, M.D.
Ernest V. Nau, M.D.

Cooperating Units: U. S. Merchant Marine Academy,
Kings Point, N. Y. 11024

Man Years:
Total: 2.3
Professional: 1.3
Other: 1.0

Project Description:

Objectives: (1) To evaluate the relative immunogenicity of two live virus vaccines (i.e., Smallpox and Yellow Fever Vaccines) given at various intervals. (2) To evaluate and improve methods for serological testing of smallpox vaccines.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings: A. Laboratory Investigations

1. Although the vaccinia plaque neutralization system assay system in rhesus monkey kidney monolayer cell cultures is now well established, there is considerable confusion in the literature as to the optimum temperature-time period for vaccinia virus neutralization: 4°C - 1 hr; room temperature - 1 hr; room temperature - 24 hr; room temperature - 4 days; 37°C - 30 min; 37°C - 70 min plus 37°C - 10 min with goat anti-rabbit γ-globulin; 37°C - 2 hr; 37°C - 2 hr followed by 4°C overnight; and 37°C - 24 hr. We were confronted with this confusion when we attempted

to select the ideal vaccinia virus neutralization test for use in our laboratory. Consequently, we were prompted to study the kinetics of vaccinia virus neutralization in a plaque assay system in order to determine the optimal temperature and length of incubation of the virus-antibody mixture.

Vaccinia virus plaque neutralization performed in 2 oz. bottle cultures of the MA-104 embryonic rhesus monkey kidney cell line showed that the neutralization of vaccinia virus with immune rabbit serum occurred optimally at 37°C for 16-24 hours.

2. Although the specificity of the vaccinia virus HI test has been confirmed by several investigators, there is some disagreement as to the optimum temperature and time of incubation of the hemagglutinin-serum mixture: room remperature - ½ hr; room temperature - 1 hr; 37° - ½ hr; 37° - 1 hr; and 37° - 2 hr. Furthermore, several host systems have been employed in the preparation of the vaccinia hemagglutinin: chorioallantoic membrane (CAM) of chicken embryos; rabbit dermis; calf dermis; sheep lymph; rabbit testis; primary rhesus monkey kidney cell culture: primary mouse embryo fibroblast culture; KB cell culture; and HeLa cell culture. Confronted with these variables when we attempted to select the ideal vaccinia virus HI system for use in our laboratory, we were prompted to study the kinetics of vaccinia virus HI test in order to determine 1) the optimal temperature and length of incubation of the hemagglutinin-serum mixture and 2) a convenient method for the preparation of the vaccinia hemagglutinin.

The optimum temperature and time of incubation of the vaccinia hemagglutination-inhibition test were 37° and 16 hr respectively. A convenient method for preparing vaccinia HA antigen consisted of infecting one-day-old primary CE fibroblasts with a high multiplicity of vaccinia virus, employing a maintenance medium devoid of serum, preparing a 20X concentration of virus-infected cells, and finally extracting the 20X antigen homogenate with genetron.

B. Volunteer Studies:

It has been generally recommended that live virus vaccines be given at least 1 month apart whenever possible. The theoretical rationale for this has been that more frequent and severe adverse reactions as well as diminished antibody responses might result. If the 1-month interval is not feasible, as with the threat of concurrent exposures or disruption of immunization programs, the vaccines should preferably be given on the same day--at different sites for parenteral products. Avoidance of the interval of about 2 days to 2 weeks has been recommended because interference between the vaccine viruses might possibly occur then.

Field observations, however, have not tended to support these theoretical considerations. Previous studies have shown that Measles and Smallpox Vaccines can be administered simultaneously, either as a single injection of combined antigens or as independent antigens at separate sites, without affecting immunogenicity as measured by seroconversions and without the potentiation of clinical reactions beyond those observed when each vaccine was given alone. Previous workers have demonstrated that live Measles, Smallpox and Yellow Fever Vaccines could be simultaneously administered safely and effectively to children.

In order to determine whether interference occurs, a study was conducted in 2 phases. In Part I, individuals were immunized initially with Smallpox Vaccine and then administered Yellow Fever Vaccine to groups simultaneously and 3, 7, 14 and 28 days later. Sera was obtained prior to and 28 days after smallpox vaccination and 28 days after yellow fever vaccination.

Part II of the study was essentially similar except that Yellow Fever Vaccine was administered initially and the time of smallpox vaccination varied as indicated above.

The results of serological tests are currently being evaluated and will be reported later.

Honors and Awards: None

Publications:

1. O'Brien, T. C., Myers, M. G., and Tauraso, N. M.: Kinetics of the vaccinia virus plaque neutralization test. Appl. Microbiol. In Press.

Serial No. 59,65,82
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on rickettsiae

Previous Serial Numbers: Same

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: Robert K. Ginn, B.S.
Edward B. Seligmann, Jr., Ph.D.

Cooperating Units: Laboratory of Control Activities, DBS

Man Years:
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: To conduct antigenic studies of rickettsial vaccines; to obtain and evaluate a reference epidemic Typhus Vaccine.

Methods Employed: Candidate reference vaccines in final containers were evaluated on the basis of (1) the standard guinea pig potency test and (2) direct complement-fixation (CF) antigen titrations. Clinical trials will be conducted with candidate reference vaccines selected on the basis of the above tests in order to evaluate their antigenic potencies in man.

Major Findings: Lots of commercially produced Typhus vaccine believed suitable for a reference vaccine have been obtained and tested by the LCA, DBS and by the manufacturer. The four candidate lots of vaccine have passed the safety, purity and potency tests ordinarily required for licensing of Typhus vaccine.

Clinical trials have been initiated to evaluate the reactogenicity and antigenicity of these preparations in volunteers.

Serial No. 59,65,82

Significance to Bio-Medical Research and Program of DBS: Selection of a reference Typhus Vaccine is essential as another means of insuring that only potent lots of new vaccine are approved and as a standard reagent for use in rickettsial research.

Proposed Course of the Project: A. This project will be continued during the coming year.

Honors and Awards:

1. Associate member (Dr. Mason), Commission on Rickettsial Diseases, Armed Forces Epidemiological Board, June 1969-June 1971.

Publications:

1. Mason, R. A. Propagation and growth cycle of *Rickettsia quintana* in a new liquid medium. *J. Bacteriol.*, 103: 184-190, 1970.

Serial No. 117
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Avian leukosis viruses in relation to human vaccines.

Previous Serial No.: Same

Principal Investigator: William G. Jahnes

Other Investigators: Calvin G. Aulisio, Ph.D.
Nicola M. Tauraso, M.D.
C. P. Li, M.D.
Lyman B. Crittenden, Ph.D.
Francis J. Judge, D.V.M.
Carl T. Hansen, D.V.M.
Amos E. Palmer, D.V.M.

Cooperating Units: Laboratory Aids Branch, DRS
Poultry Research Branch, ARS, USDA

Man Years:
Total: 1.78
Professional: 0.93
Others: 0.85

Project Description:

Objectives: (1) To test live and inactivated vaccines prepared in the chicken embryo or from chicken or duck embryo cell cultures for the presence of avian leukosis viruses (ALV); (2) to develop new and more sensitive methods of detecting viruses of the avian leukosis complex and Marek's disease; (3) to compare the relative effectiveness of the RIF (Resistance-Inducing Factor), COFAL (complement-fixation for avian leukosis) and FAB (fluorescent-antibody) tests for the detection and assay of several members of the avian leukosis complex; (4) to assist in the development of RIF-free vaccines prepared in chicken embryos or chicken and duck embryo cell cultures; (5) to determine genetic resistance to the avian leukosis viruses and to develop at the NIH Farm and Building 14G by selective cross-breeding an ALV-free chicken flock which is homogeneously susceptible to the avian leukosis viruses; (6) to continue studies on the inhibition of viral oncogenesis through the use of clam and oyster extracts and chemical inhibitors.

Methods Employed: Standard and modified cell culture techniques, complement fixation, and FAB tests were employed. Standard animal inoculation techniques were also employed.

Major Findings: 1. The testing as outlined in Objective No. 1 has been modified to include new or improved vaccines made in RIF-free chicken eggs, namely "Q" fever and epidemic typhus. A second modification as permitted in Federal Regulations was made in the testing of vaccine control fluids instead of the pre-clarified vaccines as was done previously. This latter procedure, once the seed virus was certified, circumvents the use of valuable, and sometimes low titer, antisera needed for testing the vaccines.

During this reporting period a total of 263 RIF tests were performed. Of these, 5 were tests on a total of 4 live measles vaccines; 1 was a test on a total of 1 live mumps vaccine, 2 were tests on a total of 1 live rubella vaccine, 12 were tests on a total of 4 live "Q" fever vaccines, 4 were tests on a total of 4 epidemic typhus vaccines. All were found to be RIF and FAB tests negative. Ninety-eight were tests on eggs from SPAFAS and NIH flocks. Included in the miscellaneous category were 141 tests on drugs submitted for viricidal effect, stock virus titrations, cell cultures, chicken and pigeon sera.

2. Concerning Objective No. 2, this work has been expanded to include Marek's disease along with the viruses of the avian leukosis complex in screening many different primary and continuous cell culture systems for susceptibility to these viruses (See Report #163).

3. Objective No. 3 has been a cooperative effort of the two investigators utilizing the DBS standard RIF test procedure, the fluorescent antibody test (FAB) and the COFAL test on a comparative basis. Attempts were made to prepare antibodies to several strains of RSV in two species of pigeons, and other antibody and antigen reagents were prepared and standardized for use in these studies. Other techniques were utilized as required, such as cell culture, animal and egg inoculation with viruses and tumor transplantation (See Report #164).

4. Objective No. 4 concerned studies with the RIF-free Yellow Fever Vaccine, a) evaluation of storage viability and of stabilizers, b) preparation of RIF-free Yellow Fever Vaccine in chicken embryo cell cultures (See Report #121), c) a further study was conducted to determine the comparative sensitivity of "in vivo" and "in vitro" methods of RIF virus isolation.

5. Objective No. 5 was to determine the extent of the genetic resistance to the different sub-group avian tumor viruses (A-B-C-D) in the NIH RIF-free flock and the possible correction through proper in-breeding of susceptible chickens (those whose embryos support the growth of subgroups A-B-C-D avian leukosis viruses).

A major portion of our screening effort has been to monitor the two NIH RIF-free flocks at the NIH Farm in Poolesville and on the original flock in Building 14G. Included in this screening was the aforementioned effort to correct the inbred genetic resistance to sub-group "B" (C/B) avian leukosis tumor viruses to that of genetic susceptibility to all sub-groups of avian leukosis viruses (C/O). This project was performed with the valuable collaboration of Dr. Lyman Crittenden, geneticist of USDA, Beltsville, Maryland and Drs. F. Judge, C. Hansen, and A. Palmer of the Division of Research

Services, NIH. In the flock, 2/3 of the hens were shown to be producing resistant embryos (C/B). Consequently, a system of cross-breeding had to be established, and the embryos are currently being screened for genetic status. Our efforts are being extended to determine the susceptibility to sub-groups A, B, C and D.

6. Concerning Objective No. 6, studies were conducted on the effects of paolins 1 and 2, and other chemical inhibitors on the growth and transforming ability of Rous sarcoma virus in cell cultures.

Significance to Bio-Medical Research and the Program of DBS: At present, the RIF test is routinely performed on vaccines for detecting the viruses of the avian leukosis complex. The FAB test provides a more rapid test and is a double check on vaccine testing and is also a quick and easy method for continuous monitoring of RIF-free flocks. Since RIF-free chicken embryo fibroblasts are required for the safety testing of vaccines, a RIF-free chicken flock must be maintained at NIH. The monitoring of this flock can be rapidly and easily achieved by using techniques to test eggs for maternal antibody by the indirect FAB technique. The development of more sensitive and more reliable procedures is of utmost importance to the Division's mission. The development of satisfactory tests for the agent of Marek's disease and the importance of this disease in vaccine production is essential to the Division's purpose. The maintenance of an avian tumor virus genetically susceptible RIF-free flock is of great importance for testing and for production of experimental vaccines.

Proposed Course of the Project: Work will continue on improving the present RIF, FAB, and COFAL tests and on developing simpler and more rapid procedures for the detection of the avian leukosis viruses. Studies on Marek's disease, an important cause of avian leukosis, will be continued in the hope of developing a satisfactory test system. Studies on selective inbreeding to correct resistance to susceptibility (C/0) of the NIH flock will be continued.

Our studies on avian leukosis viruses and the potential problem of their contaminating egg-derived vaccines will be reported in the future as part of a larger over-all study on endogeneous viruses contaminating avian eggs (Report #164).

Honors and Awards:

1. Invited participant/(Dr. C. P. Li), International Congress for Microbiology, Mexico City, Mexico, August 9 - 15, 1970 (Effect of L-asparaginase and Methotrexate on Rous Sarcoma Virus Focus Formation).

Publications:

1. Li, C. P., Jahnes, W. G., and Tauraso, N. M.: Studies on Inhibition of Viral Oncogenesis. II. Inhibitory Effect of L-Asparaginase, Clam Liver Extract and Methotrexate on Rous Sarcoma Virus Focus Formation. Archic. ges. Virusforsch., 32: 236-243, 1970.

Serial No. 164
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on endogenous viruses contaminating avian eggs and other cell substrates used in vaccine manufacture.

Previous Serial Number: Same

Principal Investigators: Calvin G. Aulisio, Ph.D.

Other Investigators: William G. Jahnes
Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:
Total: 1.03
Professional: 0.55
Other: 0.48

Project Description:

Objectives: A. To extend previous studies in chickens and ducks experimentally infected with Rous sarcoma virus (RSV) and other avian viruses. Antibody to these viruses are determined by the immunofluorescence test, the complement-fixation test, and the RIF test. To determine whether these viruses can be transmitted via the egg and thereby become potential contaminants in human vaccines.

B. To purify and characterize a virus causing hepatitis in ducklings.

Methods Employed: A. Indirect immunofluorescence, RIF test, complement-fixation test, and conventional cell culture techniques.

Major Findings: Individual projects (Serial Nos. 125 and 163) are reported as part of this over-all study on endogenous viruses contaminating avian eggs (Serial No. 164).

1. Mareks disease research.

Eighty percent of all chicken eggs from 20 different flocks had fluorescent antibody to Marek's herpesvirus. None of 24 eggs and 24 sera from 7 duck flocks had fluorescent antibody to Marek's herpesvirus. Numerous attempts to isolate another agent (other than herpesvirus) from tissues of Marek's virus infected chickens were unsuccessful. Although, Marek's disease is a highly contagious disease of chickens; cell-free suspensions prepared from infected tissues rarely produced the typical herpesvirus foci in cell culture (CEF, CK and DEF) and on occasion inoculated 1-day-old chicks developed typical Marek's disease symptoms but only after prolonged incubation. Attempts to isolate herpesvirus from eggs of experimentally inoculated chickens were unsuccessful. Although, a small number of eggs were examined for virus our data suggest that Marek's disease herpesvirus is not passively (egg) transmitted. Pigeons, ducks and rabbits were resistant to infection with Marek's herpesvirus--clinical symptoms and or complement-fixing and fluorescent antibodies were absent. Using a DNA inhibitor (5-BUDR) in cell cultures inoculated with cell-associated herpesvirus prevented the formation of herpesvirus foci and greatly reduced the infectivity of herpesvirus cells (CEF) inoculated into 1-day-old chicks. This limited experiment confirms the work of other investigators implicating a herpesvirus as the etiological agent of Marek's disease.

2. Subgroup C avian leukosis virus. Although, chickens experimentally inoculated with subgroup C avian leukosis virus developed low level serum antibody, eggs from several flocks tested at 1:2 dilution were negative for subgroup C antibody in the immunofluorescence test. Consequently, our previous position that subgroup C avian leukosis virus is not as prevalent in nature as subgroup A and B is further substantiated by these findings.

3. Contaminants of duck embryo fibroblast (DEF) cultures.

Examination of 50 different lots of DEF cultures failed to demonstrate any transmissible agents either by destruction of the cell sheet or when examined in the immunofluorescence test with 24 individual duck sera.

4. Contaminants of rabbit kidney cell cultures.

Examination of 21 lots of rabbit kidney cell cultures kept for 26 to 45 days and then passed weekly for 3 weeks were also negative for transmissible agents either by destruction of cell sheet or when examined by immunofluorescence with 12 individual rabbit sera and two hyperimmune (whole rabbit kidney cell suspension) rabbits.

5. Use of pigeons to produce COFAL antibody reagent.

Forty-four pigeons were inoculated with different strains of Rous sarcoma virus (RSV). The Bryan strain induced tumors in 3 of 10 birds, the Harris strain induced tumors in 2 of 6 birds, and the Schmidt-Ruppin strain induced tumors in 14 of 18 birds. Tumor size in the pigeons inoculated with Bryan and Harris viruses ranged from pea-size to 1 cm, and those in Schmidt-Ruppin virus-inoculated pigeons rangedfrom 1 to 5 cm. All pigeon sera were negative for antibody at the 1:4 dilution in the neurtalization and immunofluorescence tests.

Several of the birds had CF antibody. However, there was no correlation between tumor formation and antibody formation. Only one pigeon in each of the Bryan and Harris inoculated birds had CF antibody, but neither bird developed a tumor at the site of injection. Nine of the Schmidt-Ruppin tumor-bearing pigeons and one of the Schmidt-Ruppin non-tumor pigeons developed CF antibody.

6. Duck virus hepatitis research.

Recent acquisition of duck virus hepatitis challenge virus is presently being investigated to establish a bio-assay system for duck hepatitis virus.

Significance to Bio-Medical Research and the Program of DBS: Some licensed and experimental viral and rickettsial vaccines are prepared in chicken and duck eggs. We now have the means to study the occurrence of various microbial infections in avian flocks.

Characterization of DHV is essential for its recognition in biologicals of duck origin. Relationships to human hepatitis agents remains to be determined.

Proposed Course of Project: Studies to improve and develop immunofluorescence techniques for the detection of endogenous viruses contaminating avian eggs will continue. Since the pigeon serum is not a suitable reagent for detecting avian leukosis viruses further work with pigeons will be discontinued.

Our limited experiments suggest that Marek's herpesvirus is probably not embryo transmitted and therefore not a potential contaminant in vaccines produced in chicken embryos. Further work with Marek's herpesvirus will continue.

Honors and Awards: None

Publications: None

Serial No. 83
1. Virology & Rickettsiology
2. Cell Biology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the biology of cultured cells

Previous Serial Number: 67,79,83, and 142

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: Artrice V. Bader, Ph.D.
Jon C. Graff, M.S.
Lester E. Harris, Jr., Ph.D.
Ruth L. Kirschstein, M.D.
Richard A. Mason, D.V.M., Ph.D.
Peter G. Probst
Joseph L. Rogers
Nicola M. Tauraso, M.D.

Cooperating Units: Electron Microscope Section, Office of Coordination of Ultra-Structural Studies, Viral Oncology, NCI
Department of Chemistry, University of Maryland
Columbia Union College, Takoma Park, Maryland
Laboratory of Pathology, DBS

Man Years:
Total: 1.3
Professional: 0.9
Other: 0.4

Project Description:

Objectives: A. (1) To develop new and improved techniques and methods for large-scale production of cell cultures, (2) to prepare cell cultures for use by DBS investigators, and (3) to maintain a collection of cell lines as a repository.

B. Studies are designed to increase our knowledge of cells in culture. Some are short-term in nature and the results therefrom are immediately applicable to cell culture production problems. Other studies are pursued in more depth and are of a more fundamental nature with implications of a a broader scope. Presently, these studies are grouped under the following subheadings:

(1) Development of additional cell culture systems as a potential substrates for vaccine purposes; (2) Study of selected biochemical parameters of cells in culture as may be related to viral

susceptibility; (3) Study of factors which may influence somatic variation and modulation in cell culture systems.

Methods Employed: A. Information regarding various techniques and methodology as related to large-scale production of cells is gathered from the literature and by consultation. This information is evaluated and applied in pilot studies to the production of large quantities of cells. Upon successful application, these improved and tested techniques are standardized as part of the Cell Biology Section procedures for mass-producing cell cultures of high quality for research and vaccine testing purposes.

B. As applicable to the particular study, use is made of radioactive isotope labeling, thin layer and column chromatographic techniques, and various microscopic procedures as well as cytochemical and biochemical micromethods.

Major Findings: A. Standardization of techniques applicable to large-scale production of cell cultures.

1. The Cell Biology Section (CBS), LVR, has provided quality cell cultures to other DBS investigators (see Section C of the Laboratory Summary).

2. Upon recommendation of the Laboratory of Control Activities, DBS, trypticase soy broth (TSB) has replaced the 5% sheep blood agar as one medium employed in sterility testing. Currently, three bacteriological media are used to monitor sterility of all reagents before their use in preparing cell cultures. These include TBS, Sabouraud dextrose broth, and fluid thioglycollate incubated at both 30°C and 37°C. Of 1231 lots of cells prepared, only 1.3% were found contaminated by bacteria. Responsibility for sterility checks and reports has been given to a single individual for greater proficiency.

3. Mycoplasma testing of all cell lots frozen in the cell repository has been completed and all new lots are frozen and kept only after they have been certified as Mycoplasma-free. This year 86 lots (a total of 2794 ampoules) of Mycoplasma-free cells were frozen in liquid nitrogen. Mycoplasma-monitoring of stock cultures for production purposes is performed under contract on a weekly rather than monthly basis as heretofore done.

4. All cell cultures produced are grown with fetal calf serum which is pretested by CBS personnel for growth promoting properties and which is certified by the producer to be free from bovine viruses and Mycoplasma.

5. In conjunction with the Environmental Services Branch, NIH, work is presently under way to modify vertical laminar flow systems for large scale tissue culture production.

6. A catalog of cells in our cell bank which may be of interest to DBS investigators is being prepared.

B. Biology of cultured cells.

1. Development of Cell Substrates.

a. Reptilian cell lines. Two cell lines from snakes have successfully been developed and partially characterized. One line of cells is from a king snake kidney and the other from a fibrosarcoma of a rattlesnake. These cells are free from indigenous viral agents as shown by electron microscopy and will grown under standard conditions found optimal for mammalian cell cultures except that snake cells grow best at 39°C rather than at 37°C.

b. Vervet monkey liver cell lines. A combination of dilution and use of the micro-tissue culture plates for cell culture work has allowed us to isolate effectively from a vervet liver before culture, two quite distinct cell types. One cell was epithelial and the other a fibroblastic cell. These separate cell types were grown in the micro-plates from primary culture to 32-oz prescription bottles; frozen in liquid nitrogen and are being characterized as to some of their biologic properties. The epitheloid cell more closely resemble the heptocyte per se than any other cell now available.

c. Vervet brain tissue. Attempts are being made to apply the above techniques to isolate from host brain tissues selected cell types at the time of inoculation into cell culture.

2. Biochemical parameters of cells in culture.

a. Turnover of phospholipids of cells in tissue culture under several conditions. We are examining the turnover of phospholipids in normal human diploid cells, genetic-adherent cells, transformed cells and cells exposed to Mycoplasma and detergents by using ^{32}Pi as a label. ^{32}P-labeled phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin) and sphingomyelin from normal and Niemann-Pick diseased skin fibroblasts have been separated by column chromatography and identified by this layer chromatography. Similarities and differences in these lipid fractions are being studied on a qualitative and quantitative basis.

b. Sialic acid content of primary and continuous cell lines and its relation to viral infections. The sialic acid content of a number of continuous cell lines known for their decreased sensitivity to influenza virus infections was examined. This biochemical moiety was found to be lower in certain insensitive cells than it is in the more sensitive primary rhesus monkey kidney cells or the MDCK-USD cone "E" cell line. We are following the stability of this marker from the stage of primary culture of rhesus monkey kidney cells to that of long-term passage of the same lot of cells and will relate this to changes in the sensitivities of these cells to influenza virus infection.

3. Somatic variation and modulation in tissue culture systems.

a. Effects of trypsin. Use of trypsin as a dispersing agent in cell cultures systems appears to have a greater influence on population selection and modification than previously thought. We are conducting experiments concerned with defining more optimal conditions for the trypsinization process. One study on the time required to kill tissue culture cells by subjecting them to trypsin indicates that certain cell lines are more resistant to the killing action of trypsin than are others. Of those cell lines tested, RK-13, WI-38, and the MA-104 cell are more sensitive to tryptic killing action than the BHK-21 cell. HEp-2 and HeLa cells are intermediate in this regard.

b. A technique for dispersion of animal tissue cells was developed based on the concomitant use of trypsin and disodium ethylenediamine tetraacetate (EDTA). Use of the two dispersing agents enhances cell yield as compared with the standard cell dispersion methods. Moreover, significant reduction in the amount of time required for complete tissue dispersal, presence of a small number of nonviable cells, less cell clumpings and more uniform monolayer formation upon cultivation compare favorably with results obtained with the standard trypsinization technique.

c. Serum-free cultivation of primary animal tissue cells in vitro. Dispersed cells from the tissues of a number of animals were inoculated as primary cells into serumless tissue culture medium. Of those species tested, only cells from monkey kidneys and chick embryos were consistent in their ability to grow initially without serum as a medium supplement. A number of synthetic or chemically defined media were found to be suitable. Either whole fetal calf serum or its ablumin or globulin fraction could be used to accelerate cell growth. In addition, each form of serum protein provided adequate conditions for the growth of cells from all of the animals tested. specific protein trypsin inhibitors not of serum origin did not provide these conditions when used in place of serum proteins.

Significance to Bio-Medical Reseach and the Program of DBS: A. In order to assure reproducibility of results obtained by individuals in their control and research studies, standardization of our cell culture methodology and systems is of paramount importance.

B. Additional well-defined in vitro host systems are needed for current vaccine production and for vaccine testing. There is also a need for cell-host systems for future fastidious agents. In addition, information gained from studies in this project provides the direct and immediate basis on which changes in methodology are made in keeping with the goals of the Section in producing mass quantities of high quality cell cultures.

Proposed Course of the Project: A. Studies on standardization of techniques applicable to large-scale production of cell cultures will continue. However, these studies will be described in a separate report in the future.

B. A study of the biology of cultured cells is a continuing project and on-going investigations are developed in the light of greater knowledge, need and feasibility.

Honors and Awards:

1. Invited speaker (Dr. Orr), Department of Microbiology, University of Maryland, May 12, 1971 (Somatic variation and modulation in tissue culture systems).

Publications:

1. Montes de Oca, H., Probst, P., and Grubbs, R.: High-yield method for dispersing simian kidneys for cell cultures. Appl. Microbiol. 21: 90-94, 1971.

Serial Nos. 165, 166, 167
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of the significance and contribution of the cellular immune system in protective immunity and disease

Previous Serial Numbers: Same

Principal Investigator: Daniel E. Thor, M.D., Ph.D.

Other Investigators: Sotiros D. Chaparas, Ph.D.
Sheldon Dray, M.D., Ph.D.
Herbert J. Rapp, Ph.D.
Nicola M. Tauraso, M.D.
Sheldon M. Wolff, M.D.

Cooperating Units: University of Illinois at the Medical Center, Department of Microbiology, Chicago, Illinois
Biology Branch, NCI
Laboratory of Clinical Investigations, NIAID
Laboratory of Bacterial Products, DBS

Man Years:
Total: 1.2
Professional: 1.0
Other: 0.2

Project Description:

Objectives: A. Cellular mediated immune phenomena (delayed-type-hypersensitivity) play an important role in the pathogenesis and in immune protection of many diseases. The purpose of this project is to evaluate the relative importance of cellular and humoral factors in determining individual responses to immunization. If one or more *in vitro* procedures are found to be reliable indicators of immunity to challenge with vaccinia virus, the task of evaluating changes in the routine practice of smallpox vaccination would be aided considerably. The project was also designed to compare the cellular and humoral immune responses to revaccination reactions.

B. Another part of the study is directed toward isolation, purification and immunological characterization of cell antigens by: (1) liberating and solubilizing tissue antigens from continuous cell culture lines, diploid cell cultures, or pooled inbred animal isologous cell types, (2) purifying and isolating these antigens, and (3) preparing reagents to identify the cells and cultures from which these antigens originated.

C. Previous studies using human and guinea pig RNA species *in vitro* have shown the transfer of a delayed hypersensitivity immunological message to nonsensitive human and/or guinea pig lymphoid cells. This project is also concerned with defining the mechanism of activity, evaluating the RNA for the presence of super-antigen or super-immunogen, and testing the immunologic usefulness of RNA extracts as biologic products.

Methods Employed: Methods employed were essentially the same as those detailed in the 1969-1970 report.

Major Findings: Progress in this project was largely limited to small areas of investigation due to the serious illness of the principal investigator.

Significance to Bio-Medical Research and the Program of DBS: Of major concern in studies of delayed hypersensitivity has been the development of an *in vitro* test, particularly for man, and the discovery of the factor or factors that confer a passive hypersensitivity state. The isolation, by this laboratory of a migration inhibition factor from human lymphocytes demonstrating immunity to vaccinia virus now permits other viral studies. It has recently been reported that a definite relationship exists between "transfer factor" and the RNA extract molecules. Treatment of immunologic deficiency diseases of the cellular immune type with "transer factor" has now been used as a 'biologic product' to confer immunity to non-responders. Also, these techniques allow for the identification of tissue culture cell lines, the evaluation of culture variability and antigenicity, and the development of control reagents for the standardization and evaluation of transplantation immunology. Current cell line solubilized antigens also provide reagents for standardization and the evaluation of antilymphocyte globulin.

Proposed Course of the Project: The project will continue essentially as outlined in the antecedent report.

Honors and Awards: None

Publications:

1. Thor, D. E.: Methods in Human Migration Inhibition Factor Assay. In Bloom, B. and Glade, P. (Eds.): *Methods in Cellular Immunology*. New York, New York, Academic Press, 1971.

2. Bernstein, Irwin D., Daniel E. Thor, and Herbert J. Rapp: Tumor Immunity: Suppression of Tumor Growth *In Vitro* Initiated by Soluble Products of Specifically Stimulated Lymphocytes. *Science*, in press, 1971.

3. Chaparas, Sotiros,D., Daniel E. Thor, H. P. Godfrey, Harold Baer, and Sally R. Hedrick: Tuberculin-Active Carbohydrate That Induces Inhibition of Macrophage Migration but Not Lymphocyte Transformation. *Science*, 170: 637-639, 1970.

4. Jureziz, R. E., D. E. Thor, and S. Dray: Transfer of the Delayed Hypersensitivity Skin Reaction in the Guinea Pig Using RNA-Treated Lymphoid Cells. J. Of Immunology, 105: 1313-1321, 1970.

Serial No. 210
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the biology of continuously propagated human heteroploid cell lines.

Previous Serial Number: None

Principal Investigator: Alan V. Richman, M.D.

Other Investigators: Monroe Vincent, M.S.
Nicola M. Tauraso, M.D.

Cooperating Units: Microbiological Associates, Inc.,
Bethesda, Maryland

Man Years:
Total: .5
Professional: .4
Other: .1

Project Description:

Objectives: A. To establish an optimal system to demonstrate the malignant character of human continuous heteroploid cell lines in unconditioned experimental animals.

B. To attempt to show the presence in human heteroploid cells of hypothetical genetic material, viral or otherwise, which is related to the "transformed" continuous state in vitro.

C. As a corrolary to A and B, to establish the feasibility of using these lines derived from normal or malignant cells for vaccine production.

D. To study the immunological behaviour of these heterologous transplantation systems.

E. To examine the karyotypic stability or lability of continuously cultured human cell lines.

Methods Employed: Using "defective" virus systems applicable to other viral induced tumors in other species, we will search for a possible human cancer genome of a "helper" or a "core-like"

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nature. Hopefully, a system might be developed with concomitant development of reagents which can be applied to the human cancer problem.

Major Findings: This work is in its inception so we can only state preliminary findings. We have demonstrated that human heteroploid cells (MA-160) can show a large growth potential in experimental animals (unconditioned), leading to progressively growing tumors and death. The resultant tumor explanted cell lines have exhibited this phenomenon in a larger percentage of animals. The recovered cell lines have a morphological identity to the parent cell lines, with a diminished chromosome distribution.

Significance to Bio-Medical Research and the Program of DBS: Although human heteroploid cells can be derived from both benign and malignant sources, they have not been used for vaccine production for fear of introduction of a oncogenetic inducing agent. Experimental findings to date have not demonstrated the progressive malignant character of human heteroploid tissue culture lines in unconditioned animals. We have demonstrated the true malignant character of such cells and hope to find something which is contributing to the cytological and in vivo malignant character of such cells.

Proposed Course of Project: Depending upon further experimental findings this work will be continued at the Univ. of Pennsylvania, Department of Pathology.

Honors ans Awards: None

Publications: None

Serial No. 211
1. Virology and Rickettsiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An evaluation of the hazards by immunization based upon findings recorded in the literature will continue.

Previous Serial Number: none

Principal Investigator: J. A. Morris, Ph.D.

Coöperating Units: None

Man Years:

Total:
Professional: 1
Other 0

Project Description:

Objectives: The aim of this investigation is to determine retrospectively if full use was made of findings recorded in the scientific literature concerned with benefits and risks associated with the use in man of inactivated measles, adenovirus and influenza vaccines.

A. Measles: In 1961 an International Conference on Measles Immunization met at Bethesda to discuss and evaluate findings obtained in four years of trial use of the recently developed measles vaccines. At the first session of the Conference, attention was called (Koprowski, H., The role of hyperergy in measles encephalitis. International Conference on Measles Immunization. Bethesda, Maryland, November 7-9, 1961, pp. 103-108) to the fact that 47 years earlier an explanation of the causes of signs and symptoms in measles was published (von Pirquet, C.F., Das Bild der Masern auf ausseren Haut. 2. Kinderheilh 6:1-226,1913) which was adequate to explain the pathogenesis of measles. In the 1913 publication, hypersensitivity resulting from the interaction between specific antibody and virus antigen was described as an essential feature in measles pathogenesis.

> "The microorganisms are taken in a small quantity (in all probability through the respiratory tract); they multiply in the organism and simultaneously produce an irritation leading to the development of specific antibody which to a certain degree destroys the microorganism. The development of the antibody takes place only after 8-12 days. As soon as the antibody is present in

> greater quantities, the interaction between the microorganism and antibody leads to the formation of the toxic by-products ("apotoxine") which cause a febrile response and an inflammatory reaction on the part of the cells of the organism. . . . The fever is explained by the effect of the toxic by-products ("apotoxine") in the central organs. The petechiae result from the action of the "apotoxine on the skin and mucous membranes." (Quoted by Koprowski)

At the 1961 meeting Robbins (pp. 96-103) emphasized the role of hypersensitivity in the immune response to measles virus in man.

> "Thus it may be that hypersensitivity plays a significant role in recovery from measles infection, while at the same time it is under suspicion as contributing to some of the manifestations of illness such as skin rash and encephalitis. I am sure that few would disagree with me in suggesting that the role of hypersensitivity in virus diseases in general is a subject badly in need of further investigation."

By 1963, or within a few years after its introduction, it was established that inoculation into man of attenuated-live measles vaccine induced a mild form of measles that mimics natural disease. Injection into man of formalin-killed measles vaccine, however, brings into play risks not encountered in natural measles. First, formalin-killed measles vaccine is a poor antigen. This characteristic of the formalin-killed vaccine necessitates (a) virus concentration (by alum precipitation) and (b) repeated injections of the vaccine in the primary immunization series. These two procedures introduce for the vaccine recipient a potential danger of kidney damage (Hilleman, M.R., et al, Immunogenic response to killed measles-virus vaccine. International Conference on Measles Immunization. Bethesda, Maryland, November 7-9, 1961, pp. 274-281) and a real danger of hypersensitization to the viral components in the vaccine. It had already been recorded in 1960 that development of hypersensitivity could be expected to occur in recipients of formalin-killed vaccines of low potency. This had been noted for animals inoculated with vaccines prepared against lymphocytic choriomeningitis, Rocky Mountain spotted fever, epidemic typhus and Japanese encephalitis (Cox, H.R., In discussion: The present status of polio vaccines. Illinois Medical Journal, 118:160-168, 1960.)

The vaccinated animals were hypersensitized so that response to experimental infection was more severe than that of non-vaccinated controls. Moreover, in 1961, evidence was presented that antibody induced by formalin-killed measles vaccine might be qualitatively different from that which is induced by natural infection and therefore the vaccine-induced antibody might fail to protect against natural measles (Hilleman, M.R., loc. cit.)

With the aforementioned recorded information for the potential and real hazards associated with its use, formalin-killed measles vaccine was licensed for use in man by the Division in 1963 and in the same year the World Health Organization Scientific Group for Measles Vaccine Studies (for the composition of this Group, refer to WHO Technical Report Series No. 263. Measles Vaccines. Report of a WHO Scientific Group. WHO, Geneva, 1963.) published the following statement under the heading "Contra-indications to the Use of Measles Vaccine:"

> "(Inactivated) vaccine prepared in eggs should not be given to egg-sensitive persons. Apart from this there appear to be no contra-indications to the use of inactivated measles vaccine."

In light of this startling statement, it is little wonder that Gard (Gard, S., Virus vaccines--perspectives for the future. Conference on Cell Cultures for Virus Vaccine Production. November 6-8, 1967, pp. 575-582) in 1967 said:

> "Research in the field in immunoprophylaxis is mainly empirical. If a vaccine is found to be useful, all is well. The questions, how it works and why it is or is not effective, are seldom asked. A more rational approach to the problem of immunoprophylaxis, including systemic studies of the basic mechanisms of protective immunity, is undoubtedly to be desired."

A more rational approach to immunoprophylaxis in measles might have prevented what next developed. In 1966, reports began to appear in the literature describing serious local and systemic reactions following inoculation of attenuated-live measles vaccine in persons who had previously been injected with formalin-killed vaccine (Fulginiti, V.A., et al, Serious local reactions following live measles virus immunizations in previous killed-vaccine recipients. Journal of Pediatrics, 69:891-893, 1966.), a procedure which was advocated by the Committee on the Control of Infectious Diseases of the American Academy of Pediatrics (American Academy of Pediatrics. Schedule of Measles Vaccination: Report of the Committee on the Control of Infectious Diseases. Fifteenth edition. The Academy. Evanston, Illinois, 1966, pp. 65-70.) The 1966 report was soon followed by the publication of similar reports (see publications by Krugman, Scott, Bush, Harns) as well as by others describing severe illness following exposure to natural measles in children previously injected with formalin-killed measles vaccine (see publications by Nader, Fulginiti, Harns). Each of the published accounts was recorded as a manifestation of hypersensitization induced by injection of formalin-killed measles vaccine which, in turn, had resulted from an interaction of vaccine induced antibody and measles virus antigen.

So it appears that one has come full circle. Events which, on careful reading of a 1913 report, and brought to the attention of those gathered at the International Conference on Measles Immunization, could have been predicted to occur, have indeed come to pass and have continued to occur until now. Still, in 1970 formalin-killed measles vaccine is a licensed biologic for use in man.

B. Influenza: As early as 1944 evidence began to be accumulated that suggested that use of influenza vaccine was not accompanied with a significant reduction in susceptibility to influenza virus infection. In instances where reduction in susceptibility did occur, its persistence was of very short duration, usually 2-12 months (Commission on Influenza, AFEB, Preliminary Report. Journal of American Medical Association, 124:982-985, 1944). It was on the bases of this finding that the practice of annual vaccination against influenza was begun and has continued to the present day. This practice came into general use in spite of solid evidence gathered in 1944 from experimental studies in man that showed four months after clinically diagnosed influenza induced by inhalation of influenza virus, a third of the people involved in the study exhibited illness again when exposed to the same virus (Francis, T., Immunity in human subjects artificially infected with influenza virus, Type B., American Journal of Public Health 34:317-334, 1944) and other observers (Sigel, M., The recurrence of influenza A prime in a boarding school after 2 years. Journal of Immunology 64:33-38, 1950) identified second bouts of naturally acquired influenza within a year after an attack by a closely related virus. A vaccine can hardly be expected to induce a greater degree of protection against influenza than that which follows naturally acquired disease.

Influenza virus infection attacks superficial tissue-essentially extravascular--and consequently circulating antibody, whether induced by natural disease or by vaccination,is of limited value in preventing repeated attacks of the disease. The immune response in man to influenza virus, whether encountered in natural disease or by vaccination, puts severe theoretic limits upon benefits to be derived from parenteral infection of an inactivated influenza vaccine. This has been known for 20 years. Yet, during the period 1950-1970, increasing amounts of influenza vaccine were produced and injected into millions of people. It was not until 1964 when Langmuir mentioned his observations in a public forum that serious doubts began to be expressed openly that influenza vaccine had given even a little, if any, protection against clinical influenza (Langmuir, A. An epidemiological basis for the control of influenza. American Journal of Public Health 54:563-571, 1964) to the many millions of people inoculated with this biologic. For his evaluation of the benefits derived from the use of influenza virus vaccine, Lamgmuir, was castigated, even in a textbook (Francis, T., In Viral and Rickettsial Infection in Man. Ed. Horsfall, F. and Tamm, I., J.B. Lippincott Co., New York, Fourth Edition, 1965, p. 724) by the proponents of influenza vaccine. It is important to note that Langmuir's observations were confirmed in work carried out in Bethesda in 1963-1966 (Morris, J. A., et al., Immunity to influenza as related to antibody levels. New England Journal of Medicine 274:527-535, 1966; Brown, P., et al. Epidemic A_2 influenza in isolated Pacific island populations. American Journal of Epidemiology, 83:176-188, 1966) and in Atlanta in 1968-1969 (Dowdle, W.R., et al. Studies with inactivated influenza vaccines. WHO Bulletin, 41:355-748, 1969).

It is indeed astounding that after 34 years of use involving the inoculation into man of tens of millions of vaccine doses and the expenditure by the public of hundreds of millions of dollars, it is now recognized that the recipients of this vaccine derived little or no benefit. In 1968-1969, about 22 million vaccine doses were produced by influenza vaccine manufacturers in the United States. If one assumes that 20 million doses were used at an average cost of $5 for each injection, the resulting expenditure is $100 million.

Equally astounding are the obstacles that have been placed in the path of investigators in the Division of Biologics Standards who were assigned the task of accumulating information on which a definitive judgment might be based to determine the degree of benefit resulting from the use of influenza vaccine. When analysis of the accumulated data began to suggest that little benefit was derived from its use, all records which led to the conclusion were confiscated.

Not only has there been little or no benefit from the use of influenza vaccine in man, but harm has resulted. The vaccine contains a considerable concentration of virus material which, although inactivated, possesses toxic properties, and, if given in sufficient quantity, may cause illness in an appreciable percentage of people, especially children. Moreover, until recently, influenza vaccine contained (and still does in some instances) extraneous bacteria which could have been removed during the manufacturing process, but was not, because this was not required by the Division of Biologics Standards. These too, when injected into man, cause illness. Further, the vaccine contains antigens of chicken-egg origin which may lead to sensitization, or, rarely, to marked reactions in persons already hypersensitive to such material. In addition, the egg proteins contain blood group antigens which, when injected into man, induce the formation of specific antibody directed against that antigen. In pregnant women, this can be a dangerous event and result in fetal damage. It is for this reason that pregnant women were removed from the "high risk group" (CDC Report 1962 and compare with CDC Report 1969). It will never be known with certainty whether the recommended injection of influenza vaccine into pregnant women induced fetal damage, and if it did occur, the degree and extent of the damage. What is inexcusable is that pregnant women were placed on, and removed from, the "high risk group" without first making these determinations. This risk might have been justifiable if the vaccinated woman had been protected from contracting influenza during pregnancy, but the chances are that they were not.

In addition, every inactivated myxovirus vaccine (measles, parainfluenza, and mumps) that has been studied prospectively has been shown to hypersensitize a certain percentage of vaccinated people to subsequent exposure to natural disease induced by a virus of the same type. These observations have caused apprehensions of such a grave nature that they might well serve as a basis for condemnation of all inactivated myxovirus vaccines. Yet for influenza which is caused by a myxovirus, no such prospective study has ever been performed. However, such an experiment could have been carried out on a grand scale. In 1968-1969, Hong Kong Influenza occurred in all parts of the world. In European countries, in Canada, in South America, in Africa, in New Zealand and in Australia the disease was clinically mild with no increase in deaths. Only in the United States was the outbreak characterized by severe disease associated with a large number of deaths (WHO Chronicle, Vol. 24, No. 6, June 1970, page 264). The reason for the difference is not known. And no one in an administrative position of authority has encouraged any efforts to accumulate information to base a judgment as to whether widespread vaccination as practiced in the United States might have been a factor.

C. Adenovirus Vaccines: Thirty-one different types of adenoviruses have been recovered from man. These viruses contribute little to the total respiratory infections in adult civilian populations. However, in military recruits, because of crowding associated with training, adenovirus infections are of major economic, but not of major medical, importance. Because of their economic importance (total costs of 2 or 3 days hospitilization due to

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febrile respiratory illness in thousands of recruits) intensive efforts were made to develop a vaccine for use in the military to protect troops against the frequently encountered adenovirus types (types 3, 4 and 7).

By 1956 a formalin-killed adenovirus vaccine was developed. In the ensuing 6 years, this vaccine was injected into tens of thousands--indeed millions--of United States militaty recruits. The brief span of 3 years between 1953 (establishment of etiology) and 1956 (development of vaccine) was heralded as a "record of rapid achievement in disease control" (Hilleman, M.R., Adenovirus: History and Future of a Vaccine. In Virus Inducing Cancer. Ed. W.J. Burdette, Univ. of Utah Press, pp. 377-402, Salt Lake City, Utah, 1966).

In 1960 it was learned that formalin-inactivated adenovirus vaccine was contaminated with a virus--simian virus 40 or SV40--that was not fully inactivated by the formalin treatment to which the vaccine was subjected to kill the adenovirus components (Gerber, P., et al, Inactivation of vacuolating virus (SV40) by formaldehyde. Proceedings of the Society for Experimental Biology and Medicine 108:205-209, 1961). Further, it was learned that this contaminating virus was oncogenic in hamsters (Eddy, B., Tumors produced in hamsters by SV40. Fed. Proc. 21:930-935, 1962). Soon thereafter, it was found that adenovirus themselves were oncogenic for hamsters (Trentin, J.J., et al, the quest for human cancer virus. Science 137:835-841, 1962). These findings were followed by the discovery that growth of adenoviruses in monkey kidney cells (substrate employed in vaccine manufacture) already infected with the SV40 contaminant resulted in virus hybridization of the 2 viruses to produce a new highly oncogenic virus (Huebner, R.J., et al, Induction by adenovirus type 7 of tumors in hamsters having the antibenic characteristics of SV40 virus. Proc. National Academy of Sciences, 52:1333-1340, 1964). Next it was learned that SV40 multiplied in man and that the consequences of this multiplication were unknown (Morris, J.A., et al, Clinical and serologic responses in volunteers given vacuolating virus (SV40) by respiratory route. Proceedings of the Society for Experimental Biology and Medicine, 108:56-59, 1961). Thus 4 different oncogenic viruses capable of multiplying in man--adenovirus types 3 and 7 supposedly killed by formalin treatment, SV40 and SV40-adenovirus hybrids known not to have been killed in the vaccine processing procedures--unknowingly were injected into millions of United States troops. Also in the period between 1959 and 1964 adenovirus vaccine was conbined with influenza virus vaccine for use in civilians although it had already been established that the infrequency with which adenovirus types 3, 4 and 7--virus types contained in the vaccine--are encountered in disease in civilians obviated the need to vaccinate against adenovirus in the general population. Thus, a completely unnecessary risk was inflicted upon the civilian recipients of the combined influenza-adenovirus vaccine.

When the foregoing was learned, adenovirus vaccine was withdrawn from use for both military and civilian populations.

It appears that what evolved was a hurried effort to produce a vaccine against an illness which for the individual patient is a relatively minor one and even en masse in the military is more economic in importance than it is of medical concern.

Significance to Bio-Medical Research and the Program of DBS: An evaluation of the risks and benefits associated with the use of vaccines relates directly to the mission of the Division.

Proposed Course of the Project: An evaluation of the hazards by immunization based upon findings recorded in the literature will continue.

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Annual Report of
Laboratory of Viral Immunology
Division of Biologics Standards
July 1, 1970 through June 30, 1971

Laboratory Chief's Summary of Research

The Laboratory of Viral Immunology conducts a research program that tends to be applied in nature and product or disease oriented. Our goal is to maintain a program that will meet current and anticipated needs of the Division in the area of virology. The Laboratory conducts direct research, assists in collaborative research (guiding of contract-supported activities) and is involved in the review of scientific data submitted to the Division in its capacity as a regulatory agency. These reviews deal with existing biological products, license applications for new products and investigations covered by investigational new drug exemptions. The discharge of these responsibilities involves laboratory and clinical evaluation of products as well as test development.

Certain LVI activities are highlighted in the following paragraphs:

Rubella. During the past 1-1/2 years three manufacturers were licensed to produce live rubella virus vaccines. As a result of advisory committee recommendations for prompt and extensive vaccine use there has been a massive effort resulting in the production, distribution and administration of more than 25 million doses over a period measured in months. The LVI and DBS, having anticipated this thrust, have handled the increased load of protocol review and testing of individual lots without difficulty. Likewise the Laboratory has recognized a need to maintain a research program capable of adding to the existing data relating to safety and effectiveness as the base of clinical experience has increased from approximately 100,000 (prelicense experimental use) to many millions.

This research effort has involved both direct investigation and collaborative activities with the Center for Disease Control and other organizations. The level of vaccine-associated rubella-like reactions is more precisely defined. Encouraging information pertaining to vaccine-induced protection and duration of immunity has been analyzed and published as have new data confirming the ability of the attenuated viruses to infect the placenta (as a result of inadvertent immunization during pregnancy).

Combined Vaccines. The availability of an ever increasing array of live virus vaccines has led to appreciation of the potential advantages of developing polyvalent products. This is not a matter of mere convenience but rather a mechanism by which costs can be reduced and a higher percentage of the susceptible population effectively immunized.

Modern work on combined vaccines was in part stimulated by the investigations of our group in 1962 (Meyer, H. M., Jr. et al.: Response of Volta Children to Jet Inoculation of Combined Live Measles, Smallpox and Yellow Fever Vaccines, Bull. WHO 30:783-794, 1964). Two mixtures tested in the clinical trials, measles-smallpox and measles-smallpox-yellow fever, appeared promising for use in countries still dealing with the problems of endemic smallpox and yellow fever. Additional research by an American pharmaceutical group led to license of a combined live measles-smallpox vaccine in 1967.

More recently the development of a live mumps vaccine and subsequently a live rubella vaccine made it possible to explore the feasibility of preparing other combinations of attenuated viruses appropriate for more universal use. The principle theoretical obstacles are that multiple simultaneous infections might either enhance the reactivity of one or more components or decrease the immunizing effectiveness through viral interference (Meyer, H. M., et al.: Combined Measles-Smallpox and Other Vaccines, First International Conference on Vaccines Against Viral and Rickettsial Diseases of Man, Washington, D.C., Nov. 7-11, 1966, PAHO and WHO Scientific Publication No. 147, 336-342, May, 1967). Anticipating the course of pharmaceutical research we have maintained an active laboratory program in this area and recently conducted a series of clinical trials of products proposed for license. The overall data generated by the manufacturer, ourselves and other investigators proved the safety and potency of this second generation of polyvalent products. Thus, measles-rubella, mumps-rubella and measles-mumps-rubella vaccines were licensed during the current year. We anticipate extensive use of these new products in the American pediatric population.

Hepatitis. The LVI has a major commitment to the study of this virus-induced syndrome. Great strides have been taken over the past year in developing methods of assaying the hepatitis associated antigen (HAA) of long incubation period hepatitis ("serum hepatitis"). Likewise new techniques now make it possible to detect anti-HAA antibodies in the majority of persons infected.

Investigations supported by a DBS contract have evaluated the significance of HAA positively in donor blood in New Jersey. In these studies the sensitivity and specificity of antigen assay procedures have been defined by following the recipients of tested blood for the development of hepatitis. More than 200,000 donor units of blood have been tested with consistent evidence that commercially procured blood was more frequently HAA-positive than voluntary donor blood. The correlation of HAA with subsequent infection of the recipient was striking. Fully 69% of patients given HAA-positive units developed liver derangement or other evidence of infection as compared to only 27% of recipients of HAA-negative blood.

Translating these research findings into patient benefit has the highest priority. The first step consisted of developing standards and subsequently licensing commercial preparations for use in the detection of HAA in human blood. As these new reagents become generally available it will become possible to require the routine screening of all donor blood so as to exclude those units found to contain HAA.

To insure that testing methods had maximum sensitivity and specificity the DBS entered into a collaborative comparison of assay methods with the Committe on Plasma and Plasma Substitutes of the Division of Medical Sciences, NAS-NRC and the Center for Disease Control. These studies along with the development of standard negative and positive reagents should speed implementation and acceptance of the new techniques.

The hepatitis program has obtained other data of wide clinical, epidemiological and virological interest. For these items, such as a possible association between HAA and hepatic carcinoma, the reader is referred to the individual project report (DBS 137).

Cytomegaloviruses. These highly species-specific members of the herpes group of viruses are of considerable interest. The CMV of man are important pathogens producing maternal-fetal disease (with congenital defects) as well as other syndromes. The LVI has increased its commitment to CMV investigation during the period of this report. This work has had two objectives. The first involves the development of a subhuman primate model for study of the pathogenesis and ecology of CMV. This long-term approach will hopefully, in time provide data translatable into methods for controlling CMV disease of man.

The second aspect of the project has been to obtain information relating to the potential for contamination of poliovirus vaccines (produced in monkey kidney cell cultures) with simian CMV. Sero-epidemiological studies coupled with direct assay of monkey kidney cultures confirmed that most simians do in fact harbor small amounts of CMV. Direct tests on vaccine lots for CMV were, however, consistly negative. Subsequently experiments were conducted in which simulated vaccine was produced in cultures deliberately contaminated with CMV. The conclusions from these investigations have been that trace amounts of CMV may be present in monkey cells used for vaccine production but that infectious CMV probably rarely if ever reaches final vaccine due to its slowness of replication, cell-association and instability. Other studies have failed to detect simian CMV antibodies in monkey handlers (exposed to animals known to be excreting virus) and in recipients of polio vaccines.

While no evidence of vaccine contamination was obtained this is regarded as an undesirable situation. It should encourage the consideration of alternative cell sources for polio vaccine production.

Cell Culture Studies. Cultured human and animal cells are one of the more important tools of the medical investigator. They have diverse application in virology, oncology, genetics, etc. as well as practical uses such as the production of vaccines. Many LVI projects could be considered to be "cell culture studies". Four lines of investigation are, however, particularly appropriate for mention here.

The first concerns the extensive work of the LVI on cultures prepared from tissues of domestic and wild rabbits. This project was undertaken in 1969 in anticipation of the license of the first virus vaccine to be produced in rabbit kidney cell cultures (Cendehill strain rubella vaccine). Our investigations continued into the present year and were important in documenting the safety of the vaccine (Details can be found in individual project reports, DBS 49 and DBS 86 for the current and previous years).

A second avenue has concerned the documentation and/or development of diploid strains of human and animal cells potentially suitable for vaccine production. These investigations have been supported primarily by contract and have increased in scope during the past year. They include studies of human and animal karyology and the development and testing of diploid cell strains from monkeys and rabbits. The results to date have been promising and suggest that either human or simian diploid cells or both might be considered as possible substitutes for primary monkey cell cultures in polio vaccine production.

The third area has dealt with the question of viral etiology of rheumatoid arthritis (RA). Studies were initiated because of the observation that the joint involvement sometimes induced by rubella or rubella vaccination in many ways mimics RA. Joint fluids and synovial biopsies from RA patients were used to establish growing cells in culture. Cultures were subjected to a number of tests searching for evidence of overt or masked rubella viruses and other viruses. No evidence to date has indicated that rubella viruses bear any relationship to RA or other chronic joint disorders. (Details of the work are summarized in individual progress report DBS 183).

Finally cell cultures have been used in a wide-ranging series of genetic investigations conducted in collaboration with teams in NIMH, NHLI, NIAMD, NINDS and several universities. Determining the metabolic profile of cells cultured from patients with heritable diseases is proving useful in defining the pathological mechanisms operative in these conditions. Such studies may in time provide clues enabling prevention or therapy.

Viruses and Cancer. Over a span of years scientific attention has progressively focused on the role of viruses in the etiology of human cancer. Further progress in this area could in time lead to the development of new biological products. For this reason one component of the LVI has for several years had a full time commitment to research in viral oncology. Their work with the Epstein-Barr virus (EBV) has resulted in several exciting discoveries.

This herpes-group virus originally recovered from cultured cells derived from Burkitt's lymphoma is suspected of causing infectious mononucleosis and perhaps other lymphoproliferative diseases of man. As described in the individual progress report (DBS 127), they found that EBV infection of normal buffy coat cells in vitro led to stimulation of DNA synthesis. This characteristic has been found in animal tumor viruses but has not been associated with non-oncogenic viruses. Subsequently it was shown that EBV infection of normal lymphocytes led to transformation into continuous lymphoblastoid cells. Finally, EBV-transformed cells when inoculated into immunosuppressed newborn mice caused uncontrolled, metastasizing lymphoblastoid cell proliferation with death of the animals.

Since most humans are infected with EBV at some time during their life, these observations are of considerable interest.

LABORATORY OF VIRAL IMMUNOLOGY

Control Program

July 1, 1970 through June 30, 1971

Antibody Assays: Using virus neutralization or hemagglutination-inhibition tests.

Immune Serum Globulin (Human)

Number of lots received	140
Poliovirus potency tests	140
Number of tissue cultures	29,400
Measles potency tests (HAI)	140
Number of titrations	256

Rh_o (D) Immune Globulin (Human)

Number of lots received	28
Polio tests	28
Number of tissue cultures	5,880

Testing of Reference Materials:

NIH Measles Reference Serum No. 1

Number of tests (HAI)	31
Number of titrations	31

NIH Reference Poliomyelitis Immune Globulin Lot 174

Number of tests for polio antibodies	29
Number of tissue cultures	6,090

NIH Reference Poliomyelitis Immune Globulin Lot 175

Measles HAI tests	31
Number of titrations	31

Poliomyelitis Reference Serum IIA-4

Number of tests	16
Number of tissue cultures	4,160

Testing of Vaccines, Live, Attenuated:

Poliovirus, Oral (Screening of pre-inoculation monkey sera)

Number of neurovirulence tests	26
Number of sera	910
Number of tissue cultures	6,370

Testing of Vaccines, Inactivated:

Poliomyelitis

Number of lots received	6
Monkey potency tests	1
Pre-inoculation sera	12
Post-inoculation sera	12
Number of tissue cultures	1,440

Potency and Marker Tests (Live, Attenuated Vaccines);

Testing of Reference Materials:

A. NIH Poliovirus Reference Preparation TA_2, TB_2, TC_2	
1. Tests for Virus Content	
a. Number of tests	14
b. Number of tissue cultures	114
B. NIH Poliovirus Reference Preparations TA_3, TB_3, TC_3	
1. Tests for Virus Content	
a. Number of tests	27
b. Number of tissue cultures	972
C. NIH Po	
C. NIH Poliovirus Reference Preparations Neurovirulent	
1. Tests for Virus Content	
a. Number of tests	9
b. Number of tissue cultures	54

Testing of Vaccines, Live, Attenuated:

1. Poliovirus	
a. Monovalent lots tested for	
I. Virus Content	1
II. Genetic Marker	0
b. Trivalent lots tested for	
I. Virus Content	54
c. Number of tissue cultures	1,638
Number of tube tests	0
Number of tissue cultures	0
2. Measles	
Vaccine lots tested	12
Number titrations	24
Number of tissue cultures	768
DBS Reference	
Number titrations	16
Number of tissue cultures	512

3. Mumps

Vaccine lots tested	36
Number titrations	72
Number of tissue cultures	2,304

DBS Reference

Number titrations	31
Number of tissue cultures	992

4. Rubella

Vaccine lots tested	77
Number of tissue cultures	5,800

5. Combined Mumps - Rubella

Vaccine lots tested	4	
Mumps	8 titrations	256 tissue cultures
Rubella	3 titrations	225 tissue cultures

6. Combined Measles-Rubella

Vaccine lots tested	2	
Measles	4 titrations	128 tissue cultures
Rubella	4 titrations	128 tissue cultures

7. Combined Measles - Mumps - Rubella

Vaccine lots tested	3	
Measles	6 titrations	192 tissue cultures
Mumps	6 titrations	192 tissue cultures
Rubella	6 titrations	200 tissue cultures

Titration of DBS Rubella Virus Reference Lot 1

Number of tests	41
Number of tissue cultures	1,890

Potency Tests - Hepatitis Associated Antibody (Anti-Australia Antigen)

Total Lots tested	35
a. Counterelectrophoresis	26
b. Agar Gel Diffusion	12
c. Complement Fixation	3

Assay of Sera For Antibodies

1. Poliovirus I, II, III

Human	217
No. of tissue cultures	19,090
Horse	93
No. of tissue cultures	8,928

2. Rubella HI

Human	2,474
Guinea Pig	97
Immune Globulin (H)	193

3. Measles HI

Human	549
Monkey	12

4. Mumps - Plaque Reduction

Human	95
Control (guinea pig, human)	116
Immune Globulin	15

LABORATORY OF VIRAL IMMUNOLOGY

Production of Cell Cultures for Use in Control and Research Activities

July 1, 1970 through June 30, 1971

	Tubes	2 oz.	8 oz.	32 oz.	Petri Dishes	MT Plates
BS-C-1		416	44	18	40	
Vero		1408	120	63		69
Human Bone Marrow	103	568	33	16		
Human Leucocytes		(100 ml bottles) -320				
Human Fetal Liver		63	28			
Rabbit Kidney	1380	640	230	15		
Rabbit Liver	920	544	168	10		
Rabbit Salivary Gland	460	290	112	5		
Rabbit Spleen	460	290	112	5		
Pig Synovium		208				
BHK21		208				
MA-160	1150	120	80			
Pig Ankle	210	55	45	3		
Pig Hip	210	55	45	3		
Pig Wrist	420	110	90	6		
Pig Shoulder	210	55	45	3		
Pig Knee	210	55	45	3		
10 Chick Embryo	200	250				
RK13	8240	8	20	100		

LABORATORY OF VIRAL IMMUNOLOGY

Cell Lines Prepared and Stored in Liquid N_2

1. Total no.

Cell Lines (Tissue)	No. Lines	No. amps.
Human foreskin	1	4
embryo	1	5
prostate	1	3
bone marrow	1	6
synovium	14	49
leucocytes	29	78
spleen	1	9
amnion	1	6
fetal liver	6	35
adult liver	10	29
Heha	1	6
Rabbit spleen	14	50
liver	18	54
kidney	21	83
salivary gland	11	37
embryo	1	21
cornea	1	3
Monkey kidney	9	103
Chimp lung	1	23
liver	1	11
Baby hamster kidney	1	9
Guinea pig kidney	1	5
Pig synovium	1	3
Mouse embryo	2	6
fibroblast	1	9

Serial No. DBS 49
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning rubella virus and vaccine

Previous Serial Number: DBS 49

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators:
Hope E. Hopps, M.S.
MacGregor Conliffe, B.S.
Rudyard Wallace
Barbara C. Meyer, M.S.
Joan C. Enterline, B.A.
Robert D. Douglas
Eva R. Brown, B.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	2.1
Other:	1.4

Project Description: Part I. Studies of rubella virus infection in man.

Inadvertent rubella virus vaccination during pregnancy.

In the past year, our laboratory has been requested to perform virus studies in 9 instances in which women or adolescent girls have been inoculated with live attenuated rubella virus vaccines when pregnant or shortly before becoming pregnant. These studies have confirmed that the attenuated virus can produce chronic placental infection. None of the women had been tested for rubella susceptibility prior to vaccination; however serologic testing of later sera indicated seroconversion of one. This subject and one other reported symptoms consistent with vaccine virus infection. The attenuated virus was isolated from the conceptuses of both collected 69 and 28 days respectively after immunization. Virus isolation attempts were negative on specimens from remaining 6 women. Histopathological changes in placenta or decidua consistent with rubella infection were detected in the 2 virus-positive cases and one negative case. These data underscore the need for caution in vaccinating post-pubertal females.

Project Description: Part II. Studies directed toward the standardization of serologic tests for rubella.

At the request of the National Center for Disease Control our laboratory participated in a project to evaluate several methods for performing the rubella hemagglutination inhibition test. The variables tested included the serum treatment method (Kaolin, Dextran-sulfate-$CaCl_2$ and Heparin-$MnCl_2$) and the standard "macro" test procedure employed in our laboratory using 0.2 ml volumes of serum, antigen and indicator red cell vs. the microtiter method used at NCDC. Forty coded sera including 20 duplicate specimens were assayed. The agreement between the test methods was good; the antibody levels detected in the "macro" system were frequently 2-fold higher than those measured with the microtiter equipment. For the purposes of the trial titers were considered reproducible if the aliquots of the coded serum pair differed by no more than one 2-fold dilution. In our tests 100% reproducibility was obtained with each of the 6 techniques used.

Project Description: Part III. Studies of the antigenicity of inactivated preparations of rubella virus.

No work on inactivated preparations of rubella virus was performed during this reporting period.

Project Description: Part IV. Studies of possible rubella virus oncogenic effects.

No work on oncogenitity studies was performed during this reporting period.

Project Description: Part V. Studies of rubella virus infection in experimental animals.

No work on rubella virus infection in experimental animals was performed during this reporting period.

Project Description: Part VI. Viral and serologic studies of rabbits.

In June 1970, 3 research contracts concerning the development of rabbit diploid cell strains were awarded. In each instance the contractor is using fetal tissues from selected New Zealand rabbits (Oryctalagus). Serum specimens from all rabbits being used are sent to this laboratory where they are examined for antibody to parainfluenza 1, 2, 3; reovirus 1, 2, 3; mumps, SV-5, vaccine adeno virus, rabbit vacuolating virus, rabbit syncytial agent and rabbit herpes. During the past year, one source of rabbit procurement was rejected since sera from selected rabbits had antibody to reovirus 3. Further information concerning the development of rabbit diploid cell strains is summarized in the contract narratives.

Project Description: Part VII. Studies directed toward the standardization of potency tests for rubella-virus-vaccines.

Marked variation was encountered in the propensity of rubella-virus to produce cytopathic effects in RK_{13} cells at different cell culture passage levels. This variability has created problems in potency testing of live rubella virus vaccines submitted for release. To solve this problem 90 ampules of RK_{13} cells at the 61st DBS passage level were prepared and stored in liquid nitrogen. Cell cultures used for potency testing are currently prepared in our Section over the range of the 64th to 70th DBS passage levels. During the past year more than 8,200 cultures prepared in this manner have been produced and used in the biologics control testing of rubella virus vaccines. This modification in cell preparation has resulted in more consistent potency assays.

Project Description: Part VIII. Biologic properties of rubella virus.

a. Interferon production.

An interferon marker test for differentiating virulent and attenuated strains of rubella virus was established in 1966. Using primary green monkey kidney cell cultures (GMK), one observes low level interferon production by virulent strains while high titers are obtained with attenuated viruses. During the past year, the interferon marker has been used in the identification of 2 attenuated and 2 virulent strains of rubella virus. It is of interest that while each of these strains could be identified as virulent or attenuated in the GMK system, no striking differences in level of interferon production were measured when primary rabbit kidney cell cultures (RK) were used for interferon induction; this observation confirmed earlier studies carried out in RK_{13} cell cultures. In an attempt to find alternate systems for measuring rubella interferon, low and high passage viruses were grown in WI-38, human embryonic kidney (HEK) and human embryonic spleen (HS). While the virulent (M33, BS-C-1, 4) and a virulent (HPV-77) strains multiplied in each of the human cell types, no detectable interferon was produced. It appears that the interferon marker for rubella virus strains is quite unique since up to the present time it can only be demonstrated in a simian (GMK) cell system.

Significance to DBS Program and Bio-Medical Research: All work pertains to problems concerned with the prevention of rubella and the control of rubella vaccines.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications:

Davis, W. J., Larson, H. E., Simsarian, J. P., Parkman, P. D. and Meyer, H. M., Jr.: A Study of Rubella Immunity and Resistance to Reinfection. JAMA 215:600-608, 1971.

Meyer, H. M., Jr. and Parkman, P. D.: Rubella Vaccination, A Review of Practical Experience. JAMA 115:613-619, 1971.

Larson, H. E., Parkman, P. D., Davis, W. J., Hopps, H. E. and Meyer, H. M., Jr. Inadvertent Rubella Virus Vaccination During Pregnancy. New Eng. J. Med. , April 22, 1971.

Serial No. DBS 127
1. Laboratory of Viral Immunology
2. Section on Viral Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of viruses associated with infectious mononucleosis and human lymphoma

Previous Serial Number: 127

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators:
Davey R. Deal, M.D.
Nicholas Rogentine, M.D.
Jacqueline Whang, M.D.
Bill Hoyer, Ph.D.
Gerald Goldstein, M.D.
Berge Hampar, D.D.S.
Frank Chisari, M.D.

Cooperating Units:
National Cancer Institute
Carnegie Institute
Dept. Med. U. Virginia
Laboratory of Pathology, DBS

Man Years:

Total:	4.0
Professional:	2.0
Other:	2.0

Project Description: During the past year we concentrated our efforts mainly on studying the properties of human lymphocytes following infection with Epstein-Barr virus (EBV).

Major Findings: (1) EBV infection of mononuclear buffy coat cells of healthy donors free of detectable EBV antibodies resulted in a marked situation of cellular DNA synthesis. This reaction could be inhibited by inactivation of virus infectivity (heat or UV). Untreated control cells or cells treated with inactivated EBV lost viability after 3-4 weeks in culture. On the other hand, EBV infected cells transformed into continuous lymphoblastoid cell lines.

Only known oncogenic DNA viruses (e.g. SV_{40}, polyoma, adenovirus 12) can stimulate host DNA synthesis while non-oncogenic DNA viruses do not possess this function. It is therefore suggested that Epstein-Barr virus alone or in conjunction with other yet unknown factor(s) is involved in some human lymphoproliferation disorders. (2) When EBV-transformed lymphoblastoid cells were injected into immunosuppressed new born mice (ALS Treated) they were found to metastasize rapidly and to aggressively invade many vital organs resulting in the death of the animals. On the other hand, an equal number of fresh, peripheral leukocytes from the respective donors of the transformed cell lines, failed to grow in such immunosuppressed animals. Other lymphoid cell lines derived from both normal and malignant sources showed similar properties. It is suggested, that the EBV genome imparts these cells the properties of aggressive growth and invasiveness, commonly associated with malignancy.

Significance to DBS Program and Bio-Medical Research: Studies on viral oncogenicity are an integral part of the mission of DBS.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications:

Walsh, J. H., Gerber, P., and Purcell, R. H.: Viral Etiology after Post Perfusion Syndrom. Amer. Heart J. 80:141, 1970.

Hampar, B., Gerber, P., Hsu, K. C., Martos, L. M., Walker, J. L., Siguenza, R. F. and Wells, G. A.: Immunoferritin and Immunofluorescent Studies with Epstein-Barr Virus and Herpes Simplex Virus by Use of human sera and hyperimmune rabbit sera. J. Nat. Can. Inst. 45:75-85, 1970.

Rogentine, G. N. and Gerber, P.: Qualitation and Quantitation Comparisons of HL-A Antigens on Different Lymphoid Cell Types from the Same Individuals. Histocompability Testing, pp. 333-338, 1970.

Gerber, P. and Deal, D. R.: Epstein-Barr Virus-Induced Viral and Soluble Complement-Fixing Antigens in Burkitt Lymphoma Cell Cultures. Proc. Soc. Exp. Biol. Med. 134:748-751, 1970.

Gerber, P. and Goldstein, G.: Relationship of Epstein-Barr Virus-Induced Membrane Antigens in Lymphoid Cells to Viral Envelop Antigens. J. Immunol. 105:793-795, 1970.

Whang-Peng, J., Gerber, P. and Knutsen, T.: So-Called C Marker Chromosom and Epstein-Barr Virus. J. Nat. Can. Inst. 45:831-839, 1970.

Gerber, P., Whang-Peng, J. and Monroe, J.H.: Lymphoproliferative Effect of Epstein-Barr Virus on Normal Human Lymphocytes in Culture. Comparative Leukemia Research. pp. 739-750, 1970.

Serial No. DBS 47
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning the smallpox group of viruses and smallpox vaccines.

Previous Serial Number: DBS 47

Principal Investigator: Ruth C. Dunlap, Ph.D.

Other Investigators:
Lewellys F. Barker, M.D.
D. Gordon Sharp, Ph.D.
G. D. Washington, B.A.
H. M. Meyer, Jr., M.D.

Cooperating Units:
Department of Bacteriology & Immunology
University of North Carolina School of Medicine

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description: Part I. Basic studies of vaccinia viruses.

A. Effect of single passage in a different host system on the physical and biological characteristics of vaccinia strains.

1. Sedimentation velocity spectra. Four vaccinia strains have been studied using the BXIV zonal centrifuge rotor. These 4 strains are the DBS reference , Lot #2, a calf lymph preparation (CL), a commercial low passage chorioallantoic membrane-propagated vaccine (CAM), the CV1-78 high CAM passage strain (CV1) and the DBS reference virus passed 118 times in primary chick embryo fibroblast (PCEF) cell culture (CTC). Each of these prototype strains had a distinctive sedimentation velocity spectrum. Each strain was passed once in a host system new to it - the CAM-propagated strains were passed in PCEF cells, the CTC strain passed in CAM and the CL strain passed both in CAM and PCEF. In each case, the sedimentation velocity pattern showed a marked change. When the CAM, CV1 and CTC strains were passed back in their original host system, the sedimentation velocity spectrum shifted back towards the original pattern.

2. Dermal reactivity and neutralizing antibody production in rabbits. The CV1, CTC and CL strains and their 2 host-shift passages were inoculated intradermally into rabbits. The CV1 prototype strain caused necrotic lesions at a concentration of $10^{3.6}$ vp/ml, whereas, the CTC strain produced no necrosis

at the highest concentration used, which was $10^{8.9}$ vp, and the CL strain produced very slight necrosis at a dosage of $10^{9.7}$ vp. Passage of the CV1 strain in PCEF cells reduced its lesion-producing capacity and passage back in the CAM increased it again. When the CL and CTC strains were passed in the CAM, necrotic lesions were produced and passage in PCEF cells diminished this capacity. Propagation of these vaccinia strains in CAM seems to be associated with the production of dermal lesions in rabbits. Neutralizing antibody titers were related more to the strain of virus than to the host in which the virus was propagated. CV1 stimulated high antibody titers, CL strain produced moderate antibody levels, and the CTC strain was the least antigenic. However, each of the virus specimens employed immunized the rabbits against challenge with the CV1 strain.

3. Plaquing efficiency in MA104 cells. The CV1, CTC and CL strains and the various passages were titrated in MA104 cells. The plaquing efficiency (pfu/vp) of the CV1 strain was reduced by passage in PCEF cells and restored after back passage in CAM. The plaquing efficiency of the CL strain was greatly improved by passage in both CAM and PCEF. The CTC strain was relatively unaffected in plaquing efficiency by passage in a new host system.

B. Studies on clones of virus from the CV1-78 strain of vaccinia. Four clones isolated from CV1 by plaquing on rabbit kidney (RK) cells, are not only different from the original parent population but different from each other, in sedimentation velocity spectra and certain biological properties. Although none of these 4 populations nor the original CV1 produced any plaques on L cells, all four produced plaques after sedimentation inoculation at a multiplicity of 20 vp/cell on L cells and subsequent harvest of the progeny. L^+ RK^- clones and L^-RK^+ clones have been isolated and tested for dermal reactivity and neutralizing antibody production in rabbits. The L^+RK^- virus produces no lesions in rabbits and induces a very small neutralizing antibody response. The L^-RK^+ virus evokes necrotic lesions like the parent CV1 strain and also causes the production of high antibody titers. However, both clones protected the rabbits when challenged with high concentrations of CV1 virus.

C. Neutralization kinetics studies on vaccinia strains. The kinetics of neutralization of the CL, CAM, CV1 and CTC strains of vaccinia were measured using homologous and heterologous antisera. We found that not all of these strains are neutralized best by the homologous antisera. The CL and CAM strains were extremely refractory to neutralization by homologous antiserum although they could be neutralized by CV1 antiserum. The CV1 strain produced the highest titer of antibody and was also the most readily neutralized by any antiserum. The CTC strain was easily neutralized but induced a low antibody response. The refractoriness to neutralization evinced by the CL strain may be explained by the hypothesis that this virus may be partially neutralized already in the calf lymph preparation. When this virus is incubated with complement or anti-bovine gamma globulin, marked neutralization results, in the absence of any added antiserum. The other strains of vaccinia used in these experiments are unaffected by complement or the gamma globulin in the absence of antiserum.

D. Studies of CV1-78 vaccinia in human embryo kidney (HEK) cells. The CV1-78 strain of vaccinia does not replicate well in HEK cells. However, adsorption studies showed that virus was being taken up by the cells. Fluorescent antibody preparations indicated that viral protein was being produced in the cells to much the same extent as in control samples containing CL vaccinia which replicates well in HEK. We are pursuing this investigation by preparing ultrathin sections of HEK cells, infected with CL virus or CV1 virus, in hopes of discovering at what point in the replication cycle the development of CV1 virus is blocked.

Project Description: Part II. Clinical Studies.

There have been no clinical studies under this project this year.

Significance to Bio-Medical Research and the Program of the Division: All of this work directly pertains to research problems in the prevention of smallpox and the control of smallpox vaccines.

Proposed Course of Project: This project will be continued with special emphasis on laboratory markers for distinguishing vaccinia strains from one another. It is hoped that eventually the laboratory markers will be correlated with clinical studies in order to assist in the evaluation of proposed further attenuated or otherwise altered vaccinia strains for immunization of man.

Honors and Awards: None

Publications:

Dunlap, R. C. and Patt, J. K.: Reactivation of Heated Vaccinia Virus by UV-Irradiated Vaccinia Virus. Proc. Soc. Exp. Biol. Med. 136:1-5, 1971.

Serial No. DBS 48
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning mumps virus and vaccine

Previous Serial No.: DBS 48

Principal Investigator: Hope E. Hopps, M.S.

Other Investigators:
Robert D. Douglas
Barbara C. Meyer, M.A.
Harry M. Meyer, Jr., M.D.

Cooperating Units:

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description: Clinical studies of natural and attenuated mumps infection.

A third year survey to determine the stability of antibody in individuals at the Arkansas Children's Colony who received the Jeryl Lynn vaccine or who sustained natural mumps during the period in which the vaccine was being administered. Results of plaque neutralizing antibody tests performed in Vero cell cultures indicate that the level of neutralizing antibody remained stable during the 3rd year. Annual surveys of these populations will continue.

Significance to Bio-Medical Research and the Program of the Division: All of this work pertains directly to the control and prevention of mumps virus infection.

Proposed Course of Project: These studies will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 46
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning measles virus and vaccine

Previous Serial Number: DBS 46

Principal Investigator: Barbara C. Meyer, M.S.

Other Investigators: Hope E. Hopps, M.S.
Robert D. Douglas,
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.5
Other:	0.4

Project Description: Investigations concerning the efficacy of vaccines comprising measles-mumps-rubella, measles-rubella and mumps-rubella were undertaken. See Project Report No. DBS 126. Vaccine lots tested are reported under summary of Control Activities.

Honors and Awards: None

Publications: None

Serial No. DBS 53
1. Laboratory of Viral Immunology
2. Cell Metabolism Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study and diagnosis of heritable metabolic diseases by use of cells cultured *in vitro*.

Previous Serial Number: DBS 53

Principal Investigator: B. Wm. Uhlendorf, Ph.D.

Other Investigators:
S. Harvey Mudd, M.D.
Howard R. Sloan, M.D., Ph.D.
Elizabeth Neufeld, Ph.D.
J. Charles Pallavicini
Paul A. di Sant-Agnese, M.D.
Lynn Taussig, M.D.
Roscoe O. Brady, M.D.
Cecil B. Jacobson, M.D.
Robert Abeles, Ph.D.
Leon Rosenberg, M.D.
Stephen I. Goodman, M.D.
Leonard Warren, Ph.D.

Cooperating Units:
Laboratory of General Comparative Biochemistry, NIMH
Molecular Disease Branch, NHLI
Laboratory of Biochemistry & Metabolism, NIAMD
Pediatric Metabolism Branch, NIAMD
Laboratory of Neurochemistry, NINDS
Department of OB-GYN, George Washington Universtiy Medical Center
Department of Biochemistry, Brandeis University
Yale University School of Medicine
Department of Pediatrics, University of Colorado Medical Center
Department of Therapeutic Research, School of Medicine. University of Pennsylvania.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description: The concept which is the basis of the subprojects listed below is that a heritable metabolic disorder of an individual's metabolism may be reproduced in cultures of his cells.

Methods which are standard in tissue culture, histochemistry, chromatography, radioautography, chemical analysis, enzyme assay and radioactive tracer studies were used.

Objectives and Major Findings: (1) Cystic Fibrosis (CF): Cultured CF cells are being studied in the following ways: (a) Metabolism of glucose. The objective here is to determine the biochemical basis for the high glycogen accumulation by CF cells. (b) Microtubular proteins and how they are affected by such drugs as colchicine. (c) Characterization of the oliogosaccharide moiety of the glycoproteins of several cell fractions. So far the only positive result is that CF cells appear to respond differently than do control cells to the metabolic inhibitor ouabain.

(2) Homocystinuria: An in-depth study of the biochemical defects which produce hemocystinuria in humans is in progress. We have shown by tissue culture studies that homocystinuria is produced by a deficiency in the methylation of homocysteine to methionine as well as by the classical deficiency in the conversion of homocysteine to cystathionine. The former can be caused by a defect in the conversion of vitamin B_{12} to the form that is coenzymatically active in transmethylation. The exact position of the lesion in B_{12} metabolism is our patient's cell lines is still unknown. Our studies of the classical cystathionine synthase deficiency type of homocystinuria are concerned with the biochemical differences in cultured cells from patients with the pyridoxine-responsive type of disease on the one hand and from pyridoxine-nonresponsive patients on the other.

(3) Prenatal Diagnosis: We are continuing to make use of cultured amniotic fluid cells in making prenatal diagnoses of such diseases as Down's Syndrome, the Hurler, Hunter and San Fillipo diseases, Lesch-Nyhan disease, Tay Sachs disease, G_{M1}-gangliosidosis, homocystinuria, etc.

Significance to DBS Program and Bio-Medical Research: Human diploid cell culture is a powerful tool for the early prenatal diagnosis of heritable metabolic diseases and for studies of their underlying biochemical defects.

Proposed Course of Project: This is a continuing project.

Honors and Awards:

Invitation to participate in:

(1) The National Cystic Fibrosis Research Foundation's "GAP Conference on Cell and Tissue Culture in Cystic Fibrosis", January 22-23, 1971 at the Fogarty International Center, NIH.

(2) The 4th International Congress on Human Genetics - Round Table on "Prenatal Diagnosis of Hereditary Diseases", in Paris, France on September 6-11, 1971.

(3) The Association of Clinical Scientists' Seminar on the Clinical Pathology of Lipids, in Washington, D.C., October, 1971.

(4) Invitation to fill visiting professorship for period of one year or less starting in September 1971 in the Department of Genetics, University of Hawaii.

Publications:

Blass, J. P., Avigan, J. and Uhlendorf, B. W.: Defective decarboxylation of pyrevic acid in a child with an intermittent movement disorder. J. Clin. Invest. 49:423-432, 1969.

Pallavicini, J. C., Wiesmann, U., Uhlendorf, B. W. and di Sant-Agnese, P.: Flycogen content of tissue culture and other heritable disorders. J. Pediatrics 77:280-284, 1970.

Mudd, S. H., Uhlendorf, B. W., Hinds, K. R. and Levy, H. L.: Deranged B_{12} metabolism: studies of fibroblasts grown in tissue culture. Biochemical Med. 4:215-239, 1970.

Brady, R. O., Uhlendorf, B. W. and Jacobson, C. B.: Fabry's disease: antenatal detection. Science 172:174-175, 1971.

Serial No. DBS 137
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title:	Investigations into the etiologic agents of viral hepatitis
Previous Serial Number:	DBS 137
Principal Investigators:	Lewellys F. Barker, M.D. Michael R. Peterson, M.D. John M. Nelson, M.D.
Other Investigators:	John Thiel Harry M. Meyer, Jr., M.D. Hope E. Hopps, M.S. Roderick Murray, M.D. Ruth L. Kirschstein, M.D. Douglas E. Lorenz, Ph.D. N. Raphael Shulman, M.D. D. Gordon Sharp, Ph.D. Martin Goldfield, M.D. Stuart A. Danovitch, M.D. Howard E. Ticktin, M.D. Clarence J. Gibbs, Ph.D. Robert Cornelius, D.V.M. Robert E. Kissling, Ph.D. John Finlayson, Ph.D. Edward P. Maynard, M.D. Richard Morrow, M.D. John W. Irvin, M.D. Wade Volwiler, M.D. Henry Tenckhoff, M.D. Charles Leonard, M.D. Gail Peden, M.T. Joanna Schaefer, M.T. Barbara Fellows, R.N. Kathryn Miller, Ph.D. Charles L. Vogel, M.D.
Cooperating Units:	Office of the Director, DBS, NIH Laboratory of Pathology, DBS, NIH Laboratory of Control Activities, DBS, NIH Hematology Branch, National Institute of Arthritis and Metabolic Diseases, NIH Laboratory of Blood and Blood Products, DBS, NIH

Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina
Division of Laboratories, State of New Jersey Department of Health
Epidemiology Program and Laboratory Division, NCDC, Atlanta, Georgia
Department of Medicine, Washington Hospital Center, Washington, D.C.
George Washington University Medical Division, District of Columbia General Hospital, Washington, D.C.
Department of Medicine, Makere University College, Kampala, Uganda
USPHS Indian Health Office - Crown Point, New Mexico
Department of Medicine, Division of Gastroenterology and Division of Kidney Disease, University of Washington, Seattle, Washington
Collaborative and Field Research Unit, NINDB (NIH)
Department of Medicine, University of Kentucky, Lexington, Kentucky
University of Alabama Medical Center, Birmingham, Alabama

Man Years:

Total:	7.4
Professional:	4.5
Other:	2.9

Project Description: Part I. Evaluation of the role in acute and chronic viral hepatitis of the virus-like particles which comprise the hepatitis-associated antigen (HAA) or Australia antigen described by Blumberg and associates.

Characterization and clinical epidemiologic and immunologic studies of the virus-like particles associated with HAA have been conducted. Attempts to transmit the HAA agent to experimental animals and cell cultures are in progress.

Project Description: Part II. Search for other agents responsible for HAA negative hepatitis and chronic hepatitis.

Epidemiologic and virologic studies of HAA negative cases of hepatitis are in progress.

Objectives: The goal of these studies is the isolation and identification of infectious agents which are etiologic in viral hepatitis.

Methods Employed: Standard epidemiologic, serologic and virologic techniques are used throughout the project.

Major Findings: Part I.

A. Standards and Potency Testing of Hepatitis Associated Antibody (Anti-Australia Antigen):

Final publication of additional standards for Hepatitis Associated Antibody (Anti-Australia Antigen) in the Federal Register occurred on 30 January, 1971. The standards were prepared by members of LBBP and LVI. Potency and specificity testing of products pre and post-licensing (2 companies have been licensed to produce this product) is done in LVI (see section on Control Activities). Panels of hepatitis associated antigen (HAA) containing sera have been prepared jointly by LCA and LVI for control testing of antibody lots by manufacturers and DBS.

Efforts to improve the sensitivity and practicability of tests for HAA and for anti-HAA and to devise standards to evaluate new tests and reagents are continuing.

B. Serologic studies:

Comparison of the agar gel diffusion (AGD), counter electrophoresis (CEP) and complement fixation (CF) procedures for detection of HAA was conducted in this laboratory on large numbers of serum samples from a wide variety of sources. The relative sensitivity of the procedures was found to be AGD, CEP and CF in order of increasing sensitivity. This was confirmed in a multi-laboratory study of 120 coded serum samples. The study was a joint effort of the Committee on Plasma and Plasma Substitutes of the Division of Medical Sciences, NAS-NRC, the NCDC and the DBS. Comparison of the sensitivity of AGD, CEP and CF in 20 laboratories testing the same set of 120 coded sera gave results in agreement with those previously obtained in DBS.

The AGD, CEP and CF procedures have been used to test large numbers of sera for antibody against HAA. Currently the indirect or passive hemagglutination method is also being used to test sera for anti-HAA: it is several hundred to several thousand fold more sensitive than CEP or CF for antibody detection. In addition, we are establishing radioimmuno-assay procedures for detection of both HAA and anti-HAA. RIA would appear at present to provide the ultimate in sensitivity of the various immunologic procedures.

Serotypes of HAA, detectable with discriminating antisera in AGD, are being examined in serum collections from a number of sources. Studies are in progress to evaluate the significance of serotypes in quality control of HAA screening procedures and in immunity against HAA.

C. Clinical-epidemiologic studies:

Using the procedures for HAA and anti-HAA detection described above, we have found a high incidence of HAA in non-transfusion associated hepatitis in both East and West Africa. In addition, a provocative association between the HAA carrier state and both cirrhosis and hepatoma has been documented in East Africa. The significance of these findings is not yet clear but long-term and prospective studies are being initiated to explore the possibility of an etiologic relationship of HAA and these diseases.

Anti-HAA has been found in approximately 67% of hepatitis cases which occurred in the 1951 to 1954 studies in individuals inoculated with HAA containing materials. These antibodies have been detected by use of the passive or indirect hemagglutination method. The antibodies appear several weeks to many months after disappearance of the antigen. In a few cases of HAA positive clinical hepatitis, antibodies were detected either before inoculation or a short time after inoculation, but before the appearance of HAA and clinical hepatitis. These findings suggest that immunity from prior experience with HAA may not be completely solid. The implications for eventual vaccine development and evaluation make further assessment of the possibility of reinfection imperative.

High-risk populations including laboratory workers, blood bank workers, patients and staff in renal dialysis units, children in an institution for the mentally retarded, children in West Africa and hemophiliacs have been systematically screened for HAA and anti-HAA. In over 300 hemophiliacs the incidence of antibody is greater than 90%.

D. Electron microscopic studies:

(1) Human liver biopsies-Liver biopsies from HAA-positive and HAA-negative patients with acute viral hepatitis have been intensively studied by thin-section electron microscopy. Specimens obtained from individuals with various other non-viral liver diseases have been used as controls. Over 1800 micrographs of 36 different liver biopsies have been examined for evidence of virus particles and organelle pathology. In several HAA positive individuals with acute viral hepatitis, aggregates of 20 mμ virus-like particles were found in the nuclei of hepatic parenchymal cells. Thin sections of pellets obtained by high speed centrifugation of HAA-positive human serum are currently being examined in order to further define the relationship between the intranuclear particles and the Australia antigen.

(2) Other studies - Current projects include a) thin section examination of human gut biopsies from patients with acute viral hepatitis; b) thin section examination of liver biopsies from chimps inoculated with HAA positive material.

E. Primate studies:

Joint studies with LP, the Institute for Cancer Research, the NINDB, and Hazelton Laboratories have been directed towards assessing the susceptibility of primates to HAA infection. Rhesus, vervet and patas monkeys, including baby rhesus and vervets, have been inoculated with infectious HAA containing materials without success. Chimpanzees have been screened for HAA and anti-HAA and are now being inoculated with HAA containing sera in further attempts to establish an animal model which would be invaluable for eventual vaccine development as well as for immediate studies of virus inactivation and tissue culture studies.

F. Tissue culture studies:

Although numerous attempts have been made to establish replication of HAA in cell cultures from patients with HAA positive hepatitis as well as in a variety of primary and continuous human and primate cell lines, there are no positive findings to report in this area. Attempts are continuing to establish a specific fluorescent antibody staining method for detection of HAA in infected cells.

Part II.

Studies are continuing with LP to attempt to establish the susceptibility of marmoset monkeys to short-incubation HAA-negative hepatitis. This important question remains unresolved. Also studies have been initiated to evaluate the susceptibility of human cell cultures to an IH virus, but there are no positive findings to date.

Significance to DBS Program and Bio-Medical Research: Some of the most important unsolved problems in infectious diseases today are in the area of viral hepatitis; the work being done under this project is all pertinent to the goals of the DBS.

Proposed Course of the Project: This project will be continued.

Honors and Awards:

Dr. L. F. Barker:

(1) Consultant to *Ad Hoc* Committee on Hepatitis-Associated Antigen (HAA) Tests of the Committee on Plasma and Plasma Substitutes, Division of Medical Sciences, National Academy of Sciences-National Research Council.

(2) Consultant to the hepatitis program of the National Blood Resource Branch, National Heart and Lung Institute.

(3) Invited speaker at an Australia antigen symposium at the annual meeting of the American Association for the Study of Liver Diseases, Chicago, Ill., 5 Nov., 1970.

(4) Invited speaker in program on "Control of Hepatitis Transmission by Blood and its Components" at the Scientific Meetings of the Blood Research Institute, Boston, Mass., 20 Nov., 1970.

(5) Invited speaker at the Third Annual Meeting of the Society for Epidemiologic Research, Minneapolis, Minn., 26 June, 1970.

(6) Invited speaker at the Washington Society of Pathologists' meeting, 8 Dec., 1970.

(7) Invited speaker at a Symposium on Australia Antigen at the annual meeting of the American Gastroenterology Association, 13 May, 1971.

(8) Invited speaker on Australia Antigen at Harford Memorial Hospital, Harford, Maryland, 18 May, 1971.

Dr. Peterson:

Invited speaker on Hepatitis Associated Antigen Testing at the Annual Meeting of the Alabama Association of Medical Technologists, 20 November, 1970.

Publications:

Barker, L. F., Murray, R. and Shulman, N. R.: Viral hepatitis: studies of the hepatitis associated antigen. Military Medicine 135:374-377, 1970.

Barker, L. F., Peterson, M. R. and Murray, R.: Application of the microtiter complement fixation technique to studies of hepatitis associated antigen in human hepatitis. Vox Sanguinis 19:211-216, 1970.

Stevens, D. P. Barker, L. F., Hopps, H. E. and Meyer, H. M., Jr.: Herpes-like virus (EB virus) in continuous lymphoblastoid cell lines from patients with viral hepatitis. J. Immunol. 105:1040-1042, 1970.

Lorenz, D., Barker, L. F., Stevens, D., Peterson, M. and Kirschstein, R.: Hepatitis in the marmoset, Saguinus mystax. Proc. Soc. Exp. Biol. Med. 135:348-354, 1970.

Maynard, E. P., III, Sadikali, F., Anthony, P. B. and Barker, L. F.: Hepatitis-associated antigen and cirrhosis in Uganda. Lancet II:1326-1329, 1970.

Vogel, C. L., Anthony, P. B., Mody, N. and Barker, L. F.: Hepatitis-associated antigen in Ugandan patients with hepatocellular carcinoma. Lancet II:621-624, 1970.

Nelson, J. M., Barker, L. F. and Danovitch, S. H.: Intranuclear aggregates in the liver of a patient with serum hepatitis. Lancet II:773-774, 1970.

Serial No. DBS 52
1. Laboratory of Viral Immunology
2. Section on Viral Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Simian Virus$_{40}$

Previous Serial Number: DBS 52

Principal Investigator: Paul Gerber, Ph.D.

Cooperating Units: None

This project has been terminated.

Serial No. DBS 126
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning combined live virus vaccines.

Previous Serial Number: DBS 126

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators:	Hope E. Hopps, M.S. Barbara C. Meyer, M.S. Paul D. Parkman, M.D. Joan C. Enterline, A.B. Eva R. Brown, B.S.
Cooperating Units:	National Center for Disease Control, Virology Section and Immunization Branch, Ungulate Unit, NIH Animal Center

Man Years:

Total:	3.7
Professional:	2.5
Other:	1.2

Project Description: Part I. Potency testing procedures for combined live virus vaccines.

Combined vaccines comprising mumps-rubella, measles-mumps-rubella and measles-rubella were recently licensed. Studies of the potency testing procedures employed in the biologics control of these vaccines have been continued. In each assay, the virus under test is titrated in a suitable cell system (rubella in primary African green monkey kidney cell culture, rubeola and mumps viruses in Vero cell cultures), while the other virus or viruses are neutralized by preincubation with specific hyperimmune serum. The assays for measles and mumps components of these vaccines have been adapted to microtiter systems.

The horse antisera prepared against polioviruses types I, II and III in 1961 and used in potency assays of oral trivalent poliovirus vaccines have been depleted. Accordingly, with the assistance of the Ungulate Unit of the NIH Animal Center, ponies were immunized with poliovirus antigens prepared in our laboratory. Two animals were used for each attenuated virus type. Intravenous inoculation of 340 ml of virus antigen in 7 divided doses stimulated antibodies ranging from 1:16,384 to 1:65,536 as measured in plaque reduction tests. These sera have been shown to provide satisfactory results in assays of trivalent vaccines.

Project Description: Part II. Clinical trials of combined and simultaneously administered live virus vaccines.

The development of new live virus vaccines within the past 10 years has made possible the control of poliomyelitis; potentially the control of diseases such as measles, mumps and rubella is also feasible. These immunizing agents are all recommended to be given during infancy. Combining such vaccines or administering them simultaneously would simplify immunization schedules and would result in a considerable reduction in costs and an increase of the efficiency of vaccination programs. With this in view, a program of small clinical trials designed to provide additional information pertaining to the use of these vaccines simultaneously or in combinations has been instituted in collaboration with the National Center for Disease Control. Two projects are currently in progress. The first, on Guam, employs (1) combined Measles-Mumps-Rubella vaccines administered alone, (2) this combination given simultaneously with live oral trivalent poliovirus vaccine and (3) a comparison group given poliovirus vaccine alone. The seroconversion rates and immunogenicity of the various vaccines is currently being evaluated. Thus far, more than 160 children have been enrolled in the study and immunized by CDC personnel on Guam, and the specimens collected are being tested in our laboratory. Clinical data collected to date have indicated that the vaccines have been well tolerated.

The second project involved the use of combined live measles-rubella vaccine in children age one through 6 on the island of Tol in the Truk lagoon. A total of 355 children have been studied and pre- and post-immunization sera collected. There were no significant clinical reactions in these children as a result of immunization. Preliminary serologic results indicate that of 64 children initially seronegative for both measles and rubella virus infection vaccinated 97% developed rubella antibodies and 98% developed measles antibodies. There was no evidence suggesting that administration of these vaccines in combination resulted in interference in terms of either seroconversion rates or mean titers of antibody attained. These data confirm and extend those already available concerning the use of combined measles-rubella vaccines.

Significance to Bio-Medical Research and the Program of the Division: All of this work pertains directly to problems in the prevention and control of human infections.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 171
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning Cytomegaloviruses

Previous Serial Number: DBS 171

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators
D. Stewart Rowe, M.D.
John Bissell, M.D.
Arthur Frank, M.D.
MacGregor Conliffe, B.S.
Ronald Mayner, B.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	5.6
Professional:	5.0
Other:	0.6

Project Description: Part I. Isolation, characterization and serologic studies of cytomegaloviruses

Characterization of Simian Cytomegaloviruses (CMV).

Studies were performed to characterize viruses having the growth and cytopathic characteristics of CMV isolated from 4 rhesus and one Cercopithecus monkey. The prototype Ad-169 human strain and the CSG Cercopithecus strain were included as controls on the validity of the test procedures. Each of these viruses propagated serially in WI_{38} cells. In addition, the Cercopithecus strains were cultivated with the production of CPE in primary rabbit kidney cell cultures. Neither human nor simian viruses caused illness in intravenously inoculated adult rabbits or produced lesions on chorioallantoic membranes of embryonated chicken eggs. Acidophilic intranuclear inclusions were present in Giemsa stained infected cells; in acridine orange-stained cells these inclusions showed the green fluorescence typically produced by DNA viruses. The infectivity of both monkey and human viruses was destroyed by treatment with chloroform and passed 220 mμ pore diameter filters. Thus each of the simian isolates was shown to have characteristics consistent with those of the prototype CMV strains.

Serologic Studies of human and simian cytomegaloviruses.

Evidence for the existence of serotypes of human CMV was obtained initially from cross-neutralization tests using virus isolates and sera from infants with cytomegalic inclusion disease. Subsequent analysis of strain variation of human CMV has been hampered by the inability to produce satisfactory antisera in laboratory animals. Using techniques originally described by Hampar and his colleagues for studying serologic variations in simian CMV, rabbit antisera to human CMV have been produced in our laboratory. Thus far 7 human virus strains have been investigated. Neutralizing antibodies have been measured by a microplaque reduction technique. Cross neutralization tests using these viruses have indicated that there is considerable serologic cross-reactivity between the various strains tested. However on repeated tests reproducible 4-fold or greater differences have been shown between several of the viruses. Antisera prepared to 3 rhesus monkey CMV strains show similar patterns, suggesting that it might be possible to evaluate the biological significance of such serologic differences in experimental simian infections.

Project Description: Part II. Studies of Cytomegalovirus Infection in Experimental Animals.

Preliminary experiments to determine the characteristics of CMV infections in rhesus monkeys were performed in 3 seronegative animals maintained in isolator cages. Two animals were given virus intranasally and one was inoculated intravenously with 5,000 $TCID_{50}$ of CMV in filtered urine collected from a naturally infected rhesus monkey. The intravenously inoculated animal and one of the 2 inoculated via the nasal route developed CMV CF antibodies 14 and 28 days later. Virus was first recovered from urine and oropharyngeal swabs of both animals during the 3rd and 4th weeks post-inoculation; specimens from these animals have remained positive during the entire period during which observations have been continued, now 9 weeks following administration of virus. These data are consistent with other studies of naturally infected monkeys performed in our laboratory indicating that both oropharyngeal swab and urine specimens were persistently positive over a period of 21 months. This chronic virus shedding may be an important mechanism by which these viruses maintain cycles of infection in the wild. These simian CMV infections have many similarities to the human CMV infections occurring postnatally in man; the monkey may thus provide a useful experimental model for studies of human CMV infection.

Project Description: Part III. Studies of the epidemiology of simian cytomegalovirus infections.

Continuing studies of serum specimens obtained from both rhesus and Cercopithecus monkeys at various stages during the process of capture and delivery to laboratories in the United States have confirmed a high incidence of infection among these animals. These serologic studies indicated that CMV epidemics commonly occur among seronegative animals early in captivity under the usual system of quarantine in which large numbers of monkeys are held in open cages. It has been shown however that animals seronegative on arrival in our laboratory can be maintained free of CMV infection when quarantined individually in isolator cages.

Project Description: Part IV. Studies concerning the possible contamination of poliovirus vaccines with simian cytomegaloviruses.

Earlier studies indicated that lots of Cercopithecus kidney cell cultures were frequently contaminated with CMV. However, attempts to demonstrate such viruses as contaminents of commercially prepared monovalent bulk lots of oral poliovirus vaccines or in poliovirus harvests from known CMV contaminated primary Cercopithecus cell cultures prepared in our laboratory were unsuccessful. Quantitative studies of the virologic events occurring in BS-C-1 cell cultures inoculated first with CMV and, after this infection had become established, with attenuated poliovirus were performed. Cultures showing early focal CMV cytopathic changes were superinfected with attenuated poliovirus type I. At the time of poliovirus infection sonicated clarified homogenates of these cultures contained $10^{4.5}$ $TCID_{50}$ of CMV/1.0 ml. Poliovirus CPE destroyed the monolayers within 3 days with the production of $10^{7.8}$ $TCID_{50}$ of virus/ml. Concomitantly a progressive decline in the amount of CMV recoverable from the superinfected cultures was observed. By the 3rd day after polio was added, CMV titers were reduced by $10^{1.5}$ $\log_{10}$ $TCID_{50}$. After a single freeze-thaw cycle, levels of detectable CMV declined an additional 0.5 $\log_{10}$ units. Naturally contaminated primary Cercopithecus cultures would be expected to contain considerably less CMV at the time of seeding with poliovirus that these "artifically" CMV infected BS-C-1 cells. It seems reasonable, if the 100-fold reduction in titer observed is applicable to the situation in which primary Cercopithecus cells are used for poliovirus vaccine production, that it would be unlikely for viable CMV to survive the harvesting and multiple freeze-that cycles used in the preparation of bulk vaccines. These quantitative considerations provide a reasonable explanation for the failure to detect CMV as contaminents of poliovaccines.

Significance to DBS Program and Bio-Medical Research: These studies of agents of real or potential pathogenicity for man relate directly to the mission of the Division.

Proposed Course of the Project: Studies concerning the relationship between cytomegaloviruses and their epidemiology in human and animal host systems will be continued.

Honors & Awards: None

Publications: None

Serial No. DBS 183
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations concerning viruses as possible etiologic agents in rheumatoid arthritis

Previous Serial Number: DBS 183

Principal Investigator: Robert M. Wilkes, M.D.

Other Investigators:
Henry Roth, M.D.
Hope E. Hopps, M.S.
Barbara C. Meyer, M.A.
Harry M. Meyer, Jr., M.D.

Cooperating Units:
Arthritis Clinic, D. C. General Hospital
Washington, D. C.

Man Years:

Total:	1.9
Professional:	1.3
Other:	0.6

Project Description:

Objectives: The overall objective is to determine whether virus may play a role in the etiology of rheumatoid arthritis.

Major Findings: During the past year, in a continuing search for infectious agents in rheumatoid arthritis, we have received 13 joint fluids from patients with rheumatoid arthritis, 4 from patients with rheumatoid variants (juvenile rheumatoid arthritis and psoriatic arthritis) and 12 fluids from control patients with osteoarthritis, gout, traumatic arthritis or undefined arthritis. These fluids were inoculated on human embryonic kidney, WI-38 and primary vervet monkey kidney for virus isolation. Cells and supernatant were passed at 14 day intervals for 2 passages. These fluids were also tested for mycoplasma. No viruses or mycoplasma were isolated from any of the specimens.

Successful *in vitro* cultivation of cells was achieved with 6 of the rheumatoid fluids, 3 of the rheumatoid variants and 4 of the control fluids. 3 of these strains have been stored in liquid nitrogen.

Failure to isolate any agents from these fluids by conventional methods led to the use of the heterologous or cell fusion technique which has enjoyed limited success in virus rescue studies. Here, the synovial cells were fused with HeLa cells in the presence of betaproprolactone-inactivated Sendai virus. Heterokaryon formation is readily determined by examination of giemsa-stained coverslip preparations. The difference in morphology and tintorial properties of the synovial and HeLa cells makes it possible to distinguish and quantitate homo- and heterokaryon formation.

Technical difficulties in the heterokaryon technique have been overcome and cell cultures prepared from 2 patients with rheumatoid arthritis have been studied. To date, the cell fusion technique has not resulted in the unmasking of a latent virus.

Significance to DBS Program and Bio-Medical Research: The possible association of a virus or other microbial agent in rheumatoid arthritis makes it essential for the Division to acquire competence in this area.

Proposed Course of the Project: The project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 86
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Detection and characterization of viruses

Previous Serial Number: DBS 86

Principal Investigator: John F. Thiel

Other Investigators:
Lewellys F. Barker, M.D.
Ruth C. Dunlap, Ph.D.
Michael R. Peterson, M.D.
Hope E. Hopps, M.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units:
Laboratory of Virology & Rickettsiology
Laboratory of Pathology

Man Years:

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description:

Objectives: (1) To correlate physical and biological properties of viruses and (2) to develop more specific and more sensitive methods for detecting, identifying and classifying viruses.

Methods Employed: Cell culture techniques, electron microscopy, virus serology, fluorescence microscopy and virus infectivity titrations in tissue cultures.

Major Findings: (1) Examination of primary human kidney cell cultures for adventitious viral agents: This work has been completed and a manuscript submitted for publication.

(2) Rabbit cell cultures: fluorescence microscopy studies: Examination of rabbit tissues for the presence of adventitious agents continued with emphasis on kidney tissues from various commercial sources plus some wild American cottontails. Cells were examined using the indirect Coons reaction in which cells were tested against a battery of normal and infected sera. Further study and refinement was given to the development of reliable and efficient tests systems for routine use in detecting various rabbit viruses. Rabbit herpes and rabbit vacuolating virus test systems are reliable; the system used in testing for rabbit syncytial agent is usable if proper care is taken in di-

lution of antibody and conjugate to eliminate the tendency to non-specific fluorescence. Reo virus test systems are still under development. A similar system is under development for use in screening monkey cells for adventitious agents, especially CMV.

Significance to DBS Program and Bio-Medical Research: The development and application of methods for detecting latent agents in cell cultures is directly related to the control of vaccine production in cell cultures.

Proposed Course of the Project: This project will be continued.

Honors and Awards: None

Publications: None

Adjuncts to OD

Contracts

A

B

C

ADJUNCTS TO THE OFFICE OF THE DIRECTOR

A

B

C

Serial No. DBS 01
1. Experimental Virology
2. Office of the Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Virology

Previous Serial Number: Same

Principal Investigator: Bernice E. Eddy, Ph.D.

Other Investigators: None

Cooperating Units: None at a cost to the Government

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description:

Objectives: To investigate the safety of biological products, especially in relation to the oncogenicity of viruses and means of preventing neoplastic development; and to investigate new viruses and other agents in relation to their importance in human disease and for exploring the possibility of preventing diseases that at present are uncontrolled.

Methods Employed: Conventional methods of propagating viruses in cell cultures and in animals, and immunological methods for the detection of viral - or cellular - induced antigens or antibodies.

Major Findings: A continuing investigation in the section relates to the question of viral oncogenesis, and to the circumstances under which neoplastic changes in cells can be enhanced or supressed by an oncogenic virus or by antibodies induced by the virus or neoplastic cells.

Prior injection of hamsters with a non-oncogenic or almost non-oncogenic variant of adenovirus type 12 has been found to prevent transplantation of adenovirus type 12-induced hamster tumors under certain circumstances and to enhance the transplantation under other circumstances. The experiments are being repeated using variant virus which has been purified by gradient centrifugation.

Attempts to obtain a non-oncogenic variant of simian virus 40 by the methods used to render adenovirus type 12 non-oncogenic have been unsuccessful. Other methods for achieving success are underway.

Methods for recovering simian virus 40 from transplanted hamster tumors are being employed in attempts to recover virus from human carcinomas. Several carcinomas are under study. A virus from one has been identified as a cytomegalovirus. The role of this virus, if any, in the induction or maintenance of the carcinoma is, as yet, unknown.

With a scientist in NCI, studies of the effect of BCG on the induction of tumors is underway.

With Dr. C. P. Li in this Division, studies on the antitumor effect of clam extracts on oncogenic viruses are being continued.

Significance to Biomedical Research and the Program of the Division: This program is of major significance in developing an understanding of hazards that may exist in proposed biological products and in providing information on the importance of the relationship of immunity and oncogenesis.

Proposed Course of Project: Work will be continued on immunologic effects in relation to oncogenicity of various viruses, employing techniques and experimental protocols similar to those above but varied in different parameters.

Honors:

--On Advisory Board, American Type Culture Collection.
--Chairman, Medical Science Panel, Washington Academy of Science.
--Member of Committee on Electrons. D. C. Chapter, Sigma Xi.
--On Editorial Board of Diplomate Forum, The American Board of Medical Microbiology.
--Minor honor, photograph on the cover of the March issue of Cancer Research.

Publications:

Eddy, B. E.: Viral infectivity or oncogenesis due to viruses in hamsters. In Homburger, F. (Ed.) Hamster Pathology, S. Karger Publishing Co. Basel/New York.

Eddy, B. E., Young, R. D. and Grubbs, G. E.: Antigenic and protective efficacy of live attenuated adenovirus type 12 vaccine. Internat. Congress Microbiology 10:179, Aug. 1970.

Eddy, B. E.: Papova viruses. In Abinanti, F., et al (Eds) Standardized methods for Veterinary Microbiology. Medical Research Council. In Press.

Annual Report of the Biometrics Section
Division of Biologics Standards
July 1, 1970 through June 30, 1971

I. MISSION

The primary responsibility of the Biometrics Section, Division of Biologics Standards, is to insure that efficient statistical techniques are available and used in the scientific operations of the Division. For the most part, adequate techniques are available in the literature, but on occasion this will not be so. Even where appropriate techniques have been discussed, the literature may be scattered, highly technical, and contradictory, requiring study and comparison for adequate exploitation.

II. ANALYSES SUMMARY

In Fiscal Year 1971, 63 specific tasks were initiated in the Section and 2 were continued from the previous fiscal year. Of these, 61 were completed, 2 were cancelled or suspended, and 2 are continuing. A listing of all tasks in the Section is given in addendum.

III. COMPUTATION DEVELOPMENTS

1. A time-sharing terminal was installed in the Section on February 4, 1969, permitting an ultimate intimate incorporation of the computer into the statistical processing activities of the Section. The advantages of computer processing are limited by two considerations: (1) the extent of calculations vis a vis data input and output and (2) the availability of suitable software in the form of previously prepared programs which may be utilized with a minimum of adaptations to each specific calculation. Statistical treatment of experimental data involves a very favorable ratio of computation to input-output, but places a very heavy strain on the availability of appropriate programs. Consequently a fund of such programs must be built up over time and very sophisticated program development employed in order that the great variety of data formats can be accommodated with a minimum of program adaptations at the time of execution. Limited assistance has been obtained from commercially supplied programs; in particular, programs for inverting matrices, solving simultaneous linear regressions, polynomial equations, matrix manipulations, chi-square, and the like.

2. Principal time-sharing computer programs developed locally during this report period are: (1) a generalized two-factor analysis of variance program which obviates any need for extensive examination of the program for every change in the conditions under which it is run and with internally supplied headings, (2) a corresponding three-factor analysis of variance program, (3) a generalized missing plot program adequate for two-factor analysis of variance, (4) a linear regression

equation using matrix operations, (5) a program for calculating eigen values, (6) a program for calculating all possible t-tests under a variety of variance and sample size conditions, (7) a program for generating random experimental design treatment allocations, (8) a program which spreads multi-column tables over as many pages of teletype output as required to permit convenient and effective visual scanning of the output, (9) several "string" manipulation programs which permit supplying vitally needed legends and headings to computer calculation output, and (10) a program to calculate Magnuson's index of taxonomic affinity.

A study was made of alternatives to commercial time sharing which is currently being used in the Section. The principal alternatives are a system known as CPS (Conversational Programming Systems) available through the DCRT and a form of BASIC available through GSA. Further study of the CPS system appears to be worthwhile.

IV. METHODOLOGICAL RESEARCH

1. A correlational approach to the taxonomic classification was applied to delayed skin test response to 17 Mycobacterial strains using guinea pigs. The method yielded plausible results in agreement with accepted groupings and with a somewhat different approach due to Magnuson.

2. A fundamental examination of the concept of probability grew out of a study of the requirements for rational setting of safety standards in biologics control and elsewhere. Successive refinements of the probability concept in the past century have led to marked extension of the applicability of the probability calculations to ordinary affairs of daily living.

3. A collaborative trial of the effects of various concentrations of Tween-80 on adsorption of PPD to glass was analyzed statistically. The analysis suggested caution in acceptance of certain hypotheses based on subjective analysis of the data.

4. The influence of method of measurement, method of vaccine preparation, and route of inoculation on incidence of local and systemic reactions to vaccine injection was studied in a group of naval recruits. The analysis showed that it was necessary to examine all of the data simultaneously to achieve meaningful results.

5. A correlational approach was applied to the taxonomic classification of 17 Mycobacterial strains. While the results were gratifying, it is felt that much additional study should be undertaken.

6. Difficulties experienced by a manufacturer with assay of Polio reference standard TC3 were traced to the test procedure rather than to the reference standard.

7. A collaborative study of the adequacy of the mouse weight gain test for vaccine safety was conducted in nine cooperating laboratories. It was determined that the existing test design is on the whole satisfactory and that introduction of a standard vaccine would probably not be helpful, but it was indicated that the test might be strengthened if the data currently being obtained on controls were incorporated into the test criterion.

8. International Standard Toxoid A was compared with a new standard preparation in four strains of mice. The relative potency was found to be strain dependent.

V. OPERATIONAL PROBLEMS

Inability to furnish Division investigators with prompt, adequate and accurate statistical analyses of experimental and control data persists. This difficulty is due primarily to the fact that the Section membership has not had the experience of previous contact with an effective productive organization.

VI. FUTURE PLANS

The research activities of the Section grow out of its consulting and statistical processing activities, which cannot be anticipated. While some slight increase in the machine facilities of the Section would prove extremely helpful, in particular a Wylbur terminal and facilities for computer graphing, the primary need is to increase exploitation of existing capabilities. If the existing staff were better trained, services to the Division could be greatly increased. Development of better adapted software will be stressed.

Serial No. DBS 111
1. Office of the Director
2. Biometrics Section, DBS
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of Statistical Methodology Applicable to Biologics Control.

Previous Serial Number: DBS 111

Principal Investigator: Clifford J. Maloney, Ph.D.

Other Investigators: Harold Baer, Ph.D.; S. D. Chaparas, Ph.D. M. Carolyn Hardegree, M.D.; Thomas C. O'Brien, Ph.D.

Cooperating Units: Section on Allergenic Products; Section on Mycobacterial and Fungal Antigens; Section on Bacterial Toxins; and Section on Virology and Rickettsiology, DBS.

Man Years:

Total: 0.5
Professional: 0.3
Other: 0.2

Project Description:

Objective: As need arises from the research activity of the Division for statistical technique not adequately treated in the literature and when it appears that a need for such technique will continue on an appreciable scale in the future, theoretical and computational work is undertaken to develop appropriate technical tools.

Subproject 1: A need arose to distinguish between the delayed skin response of a number of Mycobacterial species. The literature provides a variety of conflicting procedures under the heading "multiple comparisons".

Subproject 2: Safety regulations for medical products but also many other contexts require continuous effort and expense to prevent or minimize rare but costly adverse effects, "catastrophic threats". A rational balancing of these "insurance costs" against that of the catastrophe should it occur requires estimation of very low probabilities. A study of this problem, described in the previous annual report, has led to a novel explication of the probability concept itself.

Subproject 3: The effect of Tween-80 in preventing adsorption of PPD by glass was studied in a cooperative experiment.

Subproject 4: The influence of Method of Measurement, Method of Preparation, and Route of Inoculation on incidence of local and systemic reactions to vaccine injection was studied in a group of naval recruits.

Subproject 5: Data were obtained to investigate the geometric mean skin responses of each of 17 strains of Group III Mycobacteria as a taxonomic index.

Subproject 6: Data were submitted by a manufacturer on periodic testing of TA3, TB3, and TC3 Polio reference standards.

Subproject 7: A collaborative study of the adequacy of a mouse weight gain test for vaccine safety was conducted in nine cooperating laboratories. The suitability of the use of a reference vaccine was of particular interest.

Subproject 8: International Standard Tetanus Toxoid was compared with a new preparation in four strains of mice.

Methods:

Subproject 1: Study developed that all previous approaches to "multiple comparisons" implicitly accept the n-dimensional geometry model of experimental observations, whereas an approach in the spirit of nineteenth century least squares would lead to a linear model.

Subproject 2: The logical complexities of previous attempts to explicate the concept of objective probability have been based on the limit concept which has proved so fruitful in analysis. A close study of its strengths in analysis and failures in probability suggests abandoning it in favor of an approach akin to if not identical with the recently developed non-standard analysis.

Subproject 3: The effect of Tween-80 was studied in two experiments at four concentrations and two vaccine strengths in a designed experiment that permitted analysis by analysis of variance.

Subproject 4: Four techniques were used to assay vaccine strength, which in turn was used to express frequency of local and systemic reactions as a ratio of vaccine strength. Reaction reproducibility was selected as the criterion of merit, expressed in terms of a variance and analyzed by analysis of variance.

Subproject 5: The 17 Group III strains were used to sensitize groups of Guinea pigs, and then were used to produce 17 x 17 = 289 responses. These response measures were used to produce a correlation matrix of 289 correlations. It was anticipated that closely related species should yield high correlations and less closely related species lower values. This approach permitted use of all relevant data in comparing any pair of species.

Subproject 6: Available test data were separated into readings before and readings after 1 March 1970. Separate t-tests of the difference between the before and after 1 March means were performed for each of the three strains.

Subproject 7: Analyses of variances were made on the bases of the measured mouse weight in grams.

Subproject 8: Probit analyses were calculated on the readings in each of the twelve toxoid by strain by experiment combinations. The resulting ED 50's and slopes were then analyzed by analyses of variance.

Findings:

Subproject 1: It turns out that Model I analysis of variance introduced by Fisher is inapplicable to this problem and the use of all possible t-tests, previously in vogue, is alone consistent with least squares applied to a linear model. A paper detailing these developments is being discussed with recognized experts in the field.

Subproject 2: The inadequacies of all previous attempts to develop a logically and philosophically adequate explication of the concept of probability were traced to efforts to base it on the analytical notion of a limit, which intrinsically contradicts the notion of randomness though the latter is an essential element of probability. Accepting the population probability constant as unknown and unknowable and the sample success ratio as an estimate, a distance function is next defined which yields the conventional properties of probability as conventionally understood but escapes the logical inconsistencies and incompletenesses which previous approaches suffer. A paper describing the argument in detail is being discussed with recognized leaders in the field.

Subproject 3: The results of the two experiments were not completely consistent. This may have been due in part to the fact that glass beads were used in the first experiment but omitted in the second. However, it was possible to conclude from the first experiment that Tween-80 showed a typical dose-response curve in the suppression of adsorption and from the second that no clear evidence of a physiological effect subsequent to injection was exhibited in this animal model.

Subproject 4: The four candidate measures of vaccine potency were shown to yield different levels of local and systemic reactions when all data were combined in the analyses but not when portions of the data were separately examined.

Subproject 5: The application of correlation analysis to microbial taxonomy gave results concordant with (1) the received positions of the strains and (2) a simpler technique proposed by Magnuson. There were, however, some differences and the techniques should receive additional testing.

Subproject 6: The manufacturer's claim that lower titres were in fact obtained for TC3 after than before 1 March was confirmed. However, potency was also lower in TA3 and TB3, suggesting that the result was a phenomenon of the test procedure and not of the standards.

Subproject 7: The current mouse weight gain vaccine safety test was substantially supported. In particular the investigator's question as to the desirability of a reference vaccine was answered negatively, though use of a saline control (currently run but not used in the measure) was suggested and some other details discussed.

Subproject 8: The unitage of toxoid C_2 in terms of toxoid A varied nearly twofold as determined in two strains of mice. This result, together with suggestions as to its possible explanation were transmitted to the investigator.

Significance to Bio-Medical Research and the Program of the Division:

The studies reported contributed to a marked extent to ground the Division of biologics control tests on formal statistical analysis of laboratory and field tests. The correlational approach to microbial taxonomy was found suggestive.

Honors and Awards: None

Publications:

Baer, H., Godfrey, H., Maloney, C.J., Norman, P.S., Lichtenstein, L.M.: The potency and antigen E content of commercially prepared ragweed extracts. J. Allerg. 45: 347-354, 1970

Chaparas, S.D., Maloney, C.J., and Hedrick, S.R.: Specificity of tuberculins and antigens from various species of mycobacteria. Amer. Rev. Resp. Dis. 101: 74-83, 1970

Hardegree, M.C., Barile, M.F., Pittman, M., Maloney, C.J., Schofield, F., Maclennan, R.: Immunization against neonatal tetanus in New Guinea. Bull. W. H. O. 43: 461-468, 1970

Maloney, C.J., Rastogi, S.R.: Significance test for Grubbs's estimators. Biometrics. 26: 671-676, 1970

O'Brien, T.C., Rastogi, S.R., Tauraso, N.M.: Statistical evaluation of diluents and automatic diluting and pipetting machines in influenza serology. Appl. Microbiol. 21: 311-315, 1971

O'Brien, T.C., Maloney, C.J., Tauraso, N.M.: Quantitation of residual host protein in chicken embryo-derived vaccines by radial immunodiffusion. Appl. Microbiol. 21: 1971

TABLE I

SUMMARY OF STATISTICAL ANALYSES FOR FISCAL YEAR 1971

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
253	January 1970	April 1970	Wyeth Typhoid	Dr. Feeley & Dr. Seligmann
255	February 1970	May 1970	Unsaturated Catechol	Dr. Baer
264	April 1970	Cancelled	Moving Average	Dr. Seligmann
265	April 1970	April 1970	Globulin Regression	Dr. Geller
266	April 1970	June 1970	Additions to Heat and Brain Absorption	Dr. Albrecht
267	April 1970	June 1970	Additions to Irradiation Survival	Dr. Albrecht
268	April 1970	April 1970	Incubation Time - Final Hepatitis	Dr. Barker
269	April 1970	May 1970	Skin Test	Dr. Chaparas
270	April 1970	June 1970	Magnuson Approach	Dr. Chaparas
271	April 1970	May 1970	Plaque Discrimination	Dr. Albrecht
272	April 1970	Open	Number Preference	Dr. Baer
273	May 1970	May 1970	Chi-Squares Computations	Dr. Barker
274	May 1970	July 1970	Order	Dr. Chaparas
275	May 1970	July 1970	Comant	Dr. Chaparas

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
276	May 1970	May 1970	Vaccinees with Rubella Exposure	Dr. Hopps
277	May 1970	Cancelled	Reactivity Coefficients	Dr. Godfrey
278	June 1970	October 1970	Mouse Weight Gain Test	Dr. Manclark
279	June 1970	July 1970	Weighted Regression	Dr. O'Brien
280	July 1970	July 1970	Order - 17 Chemicals	Dr. Chaparas
281	July 1970	September 1970	Vaccine Study	Dr. Martin Myers
282	July 1970	September 1970	Antigen E Precision	Dr. Baer
283	July 1970	July 1970	Geometric Means	Dr. Baer
284	August 1970	August 1970	Pyrogenic Reactions	Dr. Cohen
285	August 1970	November 1970	Mycobacteria Group III	Dr. Chaparas
286	August 1970	October 1970	Toxoid A Standards	Dr. Hardegree
287	September 1970	September 1970	Computations	Mr. Graff
288	September 1970	September 1970	Linear Regression	Mr. Graff
289	September 1970	September 1970	Polio Reference Stability	Dr. Parkman
290	September 1970	September 1970	Seasonal Effect	Dr. Baer
291	September 1970	October 1970	Grass Comparisons	Dr. Baer

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
292	September 1970	September 1970	Individual Comparisons	Dr. Maloney
293	October 1970	Open	Nonreactors	Dr. Baer
294	October 1970	November 1970	Species Titer Comparison	Dr. O'Brien
295	November 1970	November 1970	Gellec - Computer Program for Radiation Study	Dr. Geller
296	November 1970	November 1970	Microbial Systematics	Dr. Chaparas
297	November 1970	November 1970	Slope Testing	Dr. Geller
298	November 1970	January 1971	Toxoid Potency II	Dr. Hardegree
299	November 1970	March 1971	Sensitization Discrimination	Dr. Chaparas
300	November 1970	December 1970	Round Counts	Dr. Peterson
301	November 1970	December 1970	Source - Schick Toxin Potency Limits	Dr. Barile
302	December 1970	December 1970	Protein Albumin - Reproducible (I)	Dr. Cohen
303	December 1970	January 1971	Great Lakes Influenza Study	Dr. Martin Myers
304	December 1970	December 1970	Geometric Means	Dr. Baer
305	December 1970	January 1971	Human Trials	Dr. Baer
306	January 1971	May 1971	CAM Test	Dr. Fuller
307	January 1971	March 1971	Tuberculin Study	Dr. Seligmann

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
308	January 1971	February 1971	Geometric Means	Dr. Friedlander
309	February 1971	April 1971	Protein Albumin - Reproducible (II)	Dr. Cohen
310	February 1971	April 1971	Analysis of Decay Slopes	Dr. Geller
311	February 1971	May 1971	Vaccine Interference	Dr. O'Brien
312	March 1971	March 1971	CCA 11 Actual Values	Dr. O'Brien
313	March 1971	March 1971	Homologous Multiple Comparisons	Dr. Chaparas
314	March 1971	March 1971	Sample Size Estimation	Dr. Baer
315	March 1971	March 1971	Actual Values - 69C	Dr. O'Brien
316	March 1971	April 1971	Geometric Means	Dr. Friedlander
317	April 1971	May 1971	Yellow Fever Plaque	Dr. O'Brien
318	April 1971	May 1971	Smallpox Plaque	Dr. O'Brien
319	April 1971	May 1971	Collagenase Ointment	Dr. O'Malley
320	April 1971	May 1971	Smallpox Plaque - II	Dr. O'Brien
321	April 1971	April 1971	Random Numbers	Dr. Chaparas
322	April 1971	April 1971	Seasonal Effect	Dr. Baer
323	April 1971	May 1971	Collaborative Assay Study	Dr. Seligmann

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
324	April 1971	May 1971	Rubella Serum Globulins	Mrs. Brown
325	April 1971	May 1971	Kingspoint I - vaccinia	Dr. O'Brien
326	April 1971	May 1971	Kingspoint II - vaccinia	Dr. O'Brien

A

B

C

Annual Report of the Office of Investigational New Drugs
Division of Biologics Standards
July 1, 1970 - June 30, 1971

This Office has the continuing responsibility for the receipt and evaluation of "Notices of Claimed Investigational Exemption" for new biological products.

During the period covered by this report 80 "Notices" were received. This brought to 514 the total number of "Notices" received since the Food and Drug Regulations requiring submissions became effective in October 1962. Disposition of "Notices" received during the period 1963-1971 is shown in the following table:

Active	299
Withdrawn	214
Forwarded to FDA for processing	1
Total	514

The number of supplements received on active INDs throughout the year depends on the activity and number of investigators involved in the respective clinical trials. Following initial review of the "Notices," this Office has the responsibility for informing sponsors of deficiencies in the submissions. Liaison with the sponsors and the Food and Drug Administration is maintained.

The information contained in these "Notices" may indicate to the scientific staff of the Division the paths in which research should be directed for the purpose of developing Regulations for monitoring the safety and potency of new biological products. Sponsors of INDs are encouraged to submit all available data for review so that judgments as to the safety and potency can be made well in advance of the time of licensing.

The members of this Office participate actively in the scientific life of the DBS and of the National Institutes of Health attending conferences and seminars as well as representing the Division in a liaison capacity with appropriate committees of other institutes. Conferences are held periodically with representatives of manufacturers and others interested in investigational new drugs.

A

B

C

CONTRACTS PROGRAM

The contracts program is now in its fifth year of expanded operation. To date, approximately thirty-eight contracts have been completed and twenty-three are in progress.

Data obtained from many of these contracts have proven valuable by assuring the safety in humans of biological products themselves, as well as some preservatives and additives which are either in use or anticipated for use in such products. Developmental studies designed to produce subhuman primate diploid cell strains have been very successful, and candidate strains of cells are presently being distributed to all interested investigators. It appears possible that such cell strains may serve as substrates for viral vaccines in the future.

The contracts are monitored by Project Officers from the individual laboratories and by members of the Contract Staff of the Office of the Director. The scientific staff of the Division also contributes greatly to the development and function of the contracts program. This contribution has been of particular value in the initiation, supervision and evaluation of the contract efforts. Often, this has involved considerable expenditure of time, made at a sacrifice to their personal interests. Details of each contract will be found in "Contract Narratives."

A

B

C

Annual Report of the Public Information Office
Division of Biologics Standards
July 1, 1970 - June 30, 1971

I. PRESS: Licensing of hepatitis associated antibody (anti-Australia antigen, human) received wide press coverage. News release prepared and issued simultaneously with first licensee (Spectra Biologicals), was carried by Washington and New York papers, wire services, and national publications, including American Hospital Journal; JAMA; Medical World News; FD&C Reports; Drug Research Reports; Infectious Diseases; Chemical and Engineering News; American Druggist; PMA Newsletter; Good Housekeeping; McCalls; and Medical Tribune.

Licensing of antihemophilic factor (AHF)--covered by a press release prepared and issued simultaneously with release from first licensee (Stanford University) where cryoprecipitation technique was developed by Dr. Judith Pool. A statement by Dr. Pool, describing the development of the technique, was taped for DHEW Spotmaster and used by 26 radio stations. The story was carried in several medical publications including JAMA and Medical World News.

"Biologics Standards in the U.S."--featured in British Hospital Journal and Social Service Review (Sept. 1970) on DBS research and control programs. Furnished background material and photos.

Press summaries of scientific presentations by DBS investigators-- on request for press room use at two national meetings: Dr. Barker's presentation on hepatitis-associated antigen for National Academy of Sciences, and presentations by Drs. Gerber and Deal on EBV for The American Association for Cancer Research.

Ragweed extracts standardization--subject of article in Medical World News, based on interview with Dr. Baer. Photos provided for use with article.

Epstein-Barr virus studies--subject of articles for Medical World News, Medical Tribune, and Infectious Diseases (in press), based on interviews with Dr. Gerber re possible role of EB virus in malignant lympho-proliferative diseases.

Pregnancy test kits (HCG)--background furnished Detroit News and Newsday science writers researching stories on illegal interstate sale of pregnancy test kits for home use.

Pertussis vaccine--reviewed in Infectious Diseases, based on interview with Dr. Pittman.

Status of current vaccines--assisted Medical World News writer in preparation of article.

Rubella vaccine and immunization program--furnished material for Barbara Goodhart's extensive review article in Today's Health; furnished material to Reader's Digest on DBS research on vaccine strain development for inclusion in medical achievements of the past 20 years (in press); furnished copies of DBS paper on rubella reprinted from JAMA, Jan. 25, 1971, to science writers on request.

Combined live virus vaccines--at request of CDC, cooperated in preparing press release on measles-rubella and measles-mumps-rubella vaccines. Coverage in medical and trade press including wire services, Medical World News, FD&C Reports, and Drug Research Reports.

Developments prompting heavy volume of press and public inquiries: 1) statement by Dr. J. Garrott Allen suggesting the labeling of blood for transfusion from commercial sources as "high-risk;" 2) editorial in the New England Journal of Medicine by Dr. John Enders re rubella immunization program in U.S.; 3) Army approval of meningitis vaccine for military use; 4) Dr. Saul Krugman's announcement of immunization of children against hepatitis; 5) announcement by Ormont Drug & Chemical Co. of preliminary studies on Makari intradermal skin test for cancer detection.

Additional items of press interest: 1) cholera vaccine and development of cholera toxoid; 2) status of asparaginase; 3) problem re Fenwal blood bags; 4) development and availability of Zoster immune globulin; 5) safety of plastic containers for blood products; 6) Smith Kline & French Laboratories rubella vaccine recall; 7) use of human cell cultures for vaccine production; 8) licensing of frozen red blood cells; 9) regulation and control of diagnostic reagents.

Press releases required (on publication of DBS Federal Register documents)--announcements of notices of proposed standards for whole blood (modified), hepatitis associated antibody (anti-Australia antigen), and platelet concentrate; and adoption of standards for whole blood (modified). No coverage.

II. RADIO & TV: WTTG TV "Panorama"--Dr. Baer discussed poison ivy; slides were provided to illustrate appearance of plants and leaves.

WGMS "Discussion: NIH"--Dr. Chaparas interviewed by Win Clearwater on tuberculosis and BCG vaccine.

WGMS "Discussion: NIH"--Dr. Baer interviewed by Win Clearwater on house dust allergy.

III. SPECIAL PROJECTS: Summary of DBS research accomplishments for inclusion IN AAAS Report on NIH research accomplishments from 1945-1970, prepared on AAAS request to Director, NIH.

Report on DBS activities and programs prepared at request of Assistant Secretary, Community and Field Services, DHEW, for inclusion in DHEW report responding to question "Where would we be if there were no HEW?"

Awards--American Academy of Achievement's Gold Plate Award to Drs. Meyer and Parkman--biographical narrative and photos of Drs. Meyer and Parkman furnished for use in American Academy of Achievement's awards brochure. St. Lawrence

University honorary D.Sc. to Dr. Parkman--furnished St. Lawrence Univ. PR Office biographical material and photos, and assisted in preparing and distributing press material on presentation.

IV. DISTRIBUTION OF DBS PUBLICATIONS: Among many pieces of literature distributed on request were: DBS Brochure; PHS Advisory Committee on Immunization Practices recommendations; Blood and the Rh Factor; rubella fact sheet; DBS highlights of research.

V. RECURRING PROJECTS: NIH Directory-Bibliography; DBS published papers (1970 collection); NIH Almnac revision; DBS Who's Who, 1971; NIH Record stories on staff awards, appointments, retirements, etc.; NIH Calendar announcements and distribution of fliers for DBS research seminars; DBS Highlights of Research (for Congress); DHEW Annual Report; NIH Annual Report; NIH Annual Report of International Activities (DBS international activities); NIH Publications List (semi-annual revisions).

Annual Report of the Office of Scientific Communications
Division of Biologics Standards
July 1, 1970 through June 30, 1971

The responsibilities of the Office of Scientific Communications (OSC) are to plan, organize and implement appropriate programs for the collection, evaluation, storage, retrieval and distribution of the scientific and technical data and information needed for the control and research programs of the Division.

The Office continues to receive more verbal requests than written. This is a continuation of a new trend over earlier years.

Repeat requests for information from outsiders, especially from the Library of Congress, were just as great--if not greater--than last year. Many of these required further referral.

In conjunction with the Lister Hill National Center for Biomedical Communications, the Division became an experimental user of AIM-TWX, a rapid literature searching system which uses a time-sharing computer in Santa Monica, California. The enthusiasm for the service among the scientific staff was overwhelming. Over 400 AIM-TWX requests were handled this year.

Complex requests still demand extensive literature searches which usually require many personal contacts, although AIM-TWX has lessened the load considerably.

The informal network developed for sources of information continues to expand each year and now includes many private industries, professional societies, universities, hospitals, libraries and individuals, as well as other agencies of the Public Health Service and of the Federal Government. Over 500 different sources of information were consulted this year to meet the needs of the Division. An equal number of outsiders called for help.

Routinely, the OSC coordinates, indexes and distributes the Division's Annual Report.

Numerous bibliographies were compiled during this reporting period in support of decision-making conferences, for example, a selective bibliography on "Simultaneous Natural Occurring Virus Infections" and "Combined Live Virus Vaccine."

The OSC continues to have responsibility for the organization, management and effective use of the DBS library. A new procedure was implemented this year which is expected to more effectively serve the user.

The Office continues to look for better and more effective ways to meet the Division's scientific and technical information needs. Activities continue to expand as needs increase.

The following expanded master files continue to be maintained by the OSC for immediate Division use.

1. Patents involving biologicals
2. NIH Grant-sponsored research of interest to the Division's programs
3. Promotional literature involving biological products
4. World Health Organization working documents from the Expert Committee on Biological Standardization
5. World Health Organization, Technical Report Series
6. Adverse Reaction file
7. Viral Tumorigenesis research programs
8. Duplicate copies of all contracts sponsored by the Division

The Office operated this year under two serious handicaps: First, a slot vacancy since November, 1970 -- in spite of a period of high unemployment -- was not filled until May 3, 1971. Second, personnel unrest, within the Division, brought many employees into this office who required an unusual amount of time, energy, and diplomacy. In spite of these two handicaps the Office filled more requests than in any other year in its eight year history.

Contracts

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CONTRACT NARRATIVES

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CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

BIONETICS RESEARCH LABORATORIES (NIH-69-2264)

Title: Maintenance of an African Green Monkey Breeding Colony

Contractor's Project Director: Dr. David P. Martin

Project Officers (DBS): Dr. Carl E. Miller
Dr. John C. Petricciani

Objectives: To produce a number of monkey fetuses for use as needed in various projects sponsored by DBS

Methods Employed: Maintain mature, healthy African green monkeys and mate them by the best proven techniques to obtain fetuses for DBS Scientists.

Major Findings: During the past year 25 of the 30 females conceived (84%) of which 20 were live births, 5 were stillborn and none aborted. It appears that the single pair mating technique has proven very satisfactory. This involves following the menstrual cycle of each female and placing her with a male for 6 days at the appropriate time.

Significance to DBS Program and Bio-medical Research: These infants are being used to provide data on a number of projects where materials and cell lines are being tested for oncogenic potential and/or adventitious agents before being used in production of U.S. vaccines.

Proposed Course of Project: Continuation

Date Contract Initiated: June 30, 1967

Current Annual Level: $63,422

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

Allergenic Products Section

JOHNS HOPKINS UNIVERSITY (NIH-69-2010)

Title: Development of Information on the Activity of Allergenic Extracts

Contractor's Project Director: Philip Norman, M.D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer, (DBS): Mitchell H. Friedlaender, M.D.

Objectives: To determine the human reactivity of allergenic extracts of grass, ragweed pollens, and mite and house dust extracts.

Methods Employed: (1) Skin tests were carried out with extracts in sensitive humans to determine the minimum reacting doses. (2) A determination was made of the amount of each extract required to cause the release of 50% of the histamine from leucocytes of sensitive individuals. Grass pollen and mite extracts will be fractionated to isolate the active allergens.

Major Findings: With six commercial extracts of short ragweed it was shown that human reactivity was unrelated to their PNU content but was directly correlated with their Antigen E content as determined by DBS. It thus becomes possible to use a laboratory assay to measure the potency of short ragweed extracts. A number of commercial extracts of orchard and timothy grass had widely varying activities which appeared to be unrelated to their PNU content or to the concentration of a major gel diffusion band. Many, but not all, house dust sensitive individuals react to mite antigens. It appears that the mite antigen is important but not the sole antigen of house dust extracts.

Significance to DBS and Bio-Medical Research: The correlation of the human potency with a laboratory potency assay for allergenic extracts will make it possible for the first time to prescribe a potency assay for an allergenic extract. This will also help to determine whether current dating periods are suitable.

Proposed Course of Project: To continue until the objectives are attained.

Date Contract Initiated: March 1969.

Current Annual Level: $24,892

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

AMERICAN TYPE CULTURE COLLECTION (NIH-71-2126)

Title: Distribution and Storage of Diploid Animal Cells

Contractor's Project Director: Dr. John Shannon

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To store seed stock and distribution stock ampules of diploid animal cells developed by the DBS, and to distribute ampules of these cells as directed by the DBS.

Methods Employed: Cells in sealed glass ampules are stored in a government-owned liquid nitrogen refrigerator which is continuously monitored for normal operating conditions.

Major Findings: None

Significance to DBS Program and Bio-Medical Research: This project provides the DBS with a facility having a unique experience in the storage and distribution of cell cultures, and will be used for the several diploid cell lines being developed by the DBS.

Proposed Course of Project: This project will continue into fiscal year 1972.

Date Contract Initiated: May 5, 1971

Current Annual Level: $9,757

ELI LILLY LABORATORIES (NIH-69-98)

Title: Development of Sub-human Primate Diploid Cell Strains and Established Cell Lines

Contractor's Project Director: Dr. Robert N. Hull

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To develop diploid cell strains from immature male tissues of both the *Macaca mulatta* and *Cercopithecus aethiops* species of monkey which will be suitable for the production of viral vaccines for human use, and to compare the diploid cell strains with established cell lines derived from the same tissue.

Methods Employed: Tissues from selected immature male monkeys will be used to initiate cell cultures which will then be characterized with respect to growth, nutrient requirements, chromosome complement, oncogenic potential, viral susceptibility, and freedom from adventitious agents.

Major Findings: Cell cultures derived from immature male monkeys show a relatively high level of polyploidy.

Significance to DBS Program and Bio-Medical Research: The availability of a well-characterized cell system having genetic stability will allow exploration of the potential for vaccine production in cells other than primary cell cultures or human cell strains.

Proposed Course of Project: This project was terminated in fiscal year 1971 as the result of the contractor declining to submit a proposal to continue work into fiscal year 1972.

Date Contract Initiated: Approximately May 1, 1969.

Current Annual Level: $131,462.

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

MELPAR, INC. (NIH-69-2013)

Title: Electron Microscopic Examination of Cell Lines for Virus-like Particles

Contractor's Project Director: John Verna, Ph.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
John M. Nelson, M.D., LVI

Proposed Course of Project: There has been no further work under this contract since the 1970 annual report. The contract was terminated as of October, 1970.

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

UNIVERSITY OF OREGON MEDICAL SCHOOL (PH43-67-675)

Title: Investigations on the Mechanisms of Chemical Carcinogenesis

Contractor's Project Director: Dr. A. Wesley Horton

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officer (DBS): Dr. Carolyn Hardegree, LBP

Objectives: To develop short term biophysical and biological tests to identify carcinogens or promoters and to apply such tests to the components of oil adjuvants.

Methods Employed: Evaluation of mineral and vegetable oils and emulsifiers through the use of biophysical and animal tests:

(1) Ionic Transport. Measurements were made on the enhancement or inhibition of carcinogenesis by promoters and oil fractions on the transport of anionic dye stuffs (simulating metabolites of carcinogens) from an aqueous to an organic phase, mediated by phospholipids and cholesterol.

(2) Monomolecular films: Measurements were made of the surface tension of a reference monomolecular film of cholesterol compared to values obtained when carcinogens, adjuvant components and noncarcinogens were mixed with cholesterol. Test components showing unusual effects in this system were subjected to skin-painting experiments in mice to determine if the biophysical data could be confirmed.

(3) C3H and Swiss mice have been skin-painted and injected subcutaneously with oil adjuvants and their fractions to determine any direct oncogenic activity.

(4) The serum properdin levels of mice treated with adjuvant components are being measured to determine the relationship between properdin levels and promoting or oncogenic activity.

Major Findings: The ionic transport model system showed that the C18-C20 n-paraffins might be more active promoters of carcinogenesis than the C12-C14 members of the series. Skin-painting experiments in mice have confirmed this observation.

Cholesterol, a component of cell membranes, at high concentrations promotes the rate of dye transport whereas at low concentrations it retards transport.

Component No. 1, one of three oils blended to produce Drakeol mineral oil, and shown primarily responsible for the promoting activity of the oil, has also been shown to have most of the activity for inducing fibrous encapsulation of oil in mice.

A test system in mice has been developed which requires only half the time (20 weeks) previously required to determine promoting activity of carcinogenesis.

The principal component of peanut oil, triolein, has inhibiting activity for skin carcinogenesis.

Component No. 2 and a thermal diffusion fraction of mineral oil were shown to be inhibitors of skin carcinogenesis.

Significance to DBS Program and Biomedical Research: These studies are designed to develop quick biophysical tests to determine the presence of carcinogens in components of oil adjuvants; elucidate mechanisms of action of carcinogenesis and establish criteria for the safety of adjuvant preparations.

Proposed Course of Project: The contract will terminate March 31, 1972.

Date Contract Initiated: March 17, 1967

Current Annual Level: $34,540

Publication: Horton, A.W. and McClure, D.W.: Ionic transport through model membranes I. Effects of accelerators of carcinogenesis. Biochimica Biophysica Acta. In Press.

UNIVERSITY OF PITTSBURGH (PH 43-67-701)

Title: Cytogenetic Follow-up of Intrauterine and Exchange Transfused Children

Contractor's Project Director: Dr. J. Howard Turner

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To continue a prospective cytogenetic study of intrauterine and exchange transfused children by following the persistence of mitotically active transfused cells in the recipients.

Methods Employed: Periodic cytogenetic analyses using peripheral blood will be made on those children already included in the study. Less frequent general pediatric examinations will also be conducted on selected cases.

Major Findings: Persistence of donor leukocytes has been demonstrated in a limited number of children receiving intrauterine and exchange transfusions. A case of apparent *in vivo* hybridization among donor and recipient leukocytes (lymphocytes) has been detected.

Significance to DBS Program and Bio-Medical Research: The potential for establishing a foreign cell line in the fetus or newborn as the result of a transfusion will be determined, and appropriate recommendations might be made to reduce this risk.

Proposed Course of Project: No additional cases will be added after fiscal year 1971; but follow-up of selected cases may continue for several years.

Date Contract Initiated: April 27, 1967

Current Annual Level: $21,383

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA (NIH 71-2083)

Title: Spherule Coccidioidin in the Detection of Coccidioidomycosis

Contractor's Project Director: Dr. Hillel B. Levine

Project Officer (DBS): Dr. Sotiros D. Chaparas

Objectives: 1) To produce coccidioidin from spherule endospores of six selected strains of *Coccidioides immitis*. 2) Characterize the antigenic composition, chemistry, and stability of these skin reactive substances. 3) Compare the reactivity and specificity of spherule coccidioidin and mycelial coccidioidin in sensitized animals and in man. 4) To provide the Government with materials suitable for a U. S. reference coccidioidin.

Methods Employed: Cultures producing spherule endospores will be grown, extracts prepared and the extracts tested in animals and man.

Major Findings: None

Significance to DBS Program and Bio-Medical Research: This project will provide DBS with reference preparations and data on which to base the potency of coccidioidin.

Proposed Course of Project: This project will continue into fiscal year 1972.

Date Contract Initiated: April 5, 1971

Current Annual Level: $26,039

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA (NIH 71-2082)

Title: Studies on Coccidioidin Derived from Single Strains of Coccidioidin

Contractor's Project Director: Dr. Demosthenes Pappagianis

Project Officer (DBS): Dr. Sotiros D. Chaparas

Objectives: 1) To produce, under optimal conditions, coccidioidin from mycelia of six selected strains of Coccidioides immitis. 2) Characterize the antigenic composition, chemistry, and stability of these skin reactive substances. 3) Compare the reactivity and specificity of mycelial and spherule coccidioidin in sensitized animals and in man. 4) To provide the Division with materials suitable for a U. S. reference coccidioidin.

Methods Employed: Cultures of the six strains will be grown, extracts prepared and the extracts tested in animals and man.

Major Findings: None

Significance to DBS Program and Bio-Medical Research: This project will provide DBS with reference preparations and data on which to base the potency of coccidioidin.

Proposed Course of Project: This project will continue into fiscal year 1972.

Date Contract Initiated: March 30, 1971

Current Annual Level: $26,500

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

UNIVERSITY OF TEXAS (NIH-70-2247)

Title: Cytological Characterization of Sub-human Primate Cell Cultures

Contractor's Project Director: Dr. T. C. Hsu

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To establish cytogenetic characteristics of primary cell cultures derived from several tissues of the *Macaca mulatta* and *Cercopithecus aethiops* species of monkey to serve as guidelines in evaluating cytogenetic data on diploid cell lines of these species.

Methods Employed: Tissues are obtained from animals sacrificed at the DBS for kidney cell cultures and sent to Dr. Hsu's laboratory for preparation of chromosomes and evaluation.

Major Findings: None to date.

Significance to DBS Program and Bio-Medical Research: The data generated in this project will serve as guidelines in evaluating the cytogenetic characteristics of the monkey diploid cells being developed by the DBS for possible use in production of viral vaccines for humans.

Proposed Course of Project: This project will continue into fiscal year 1972.

Date Contract Initiated: Approximately May 1, 1969.

Current Annual Level: $87,700.

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

Section on Immunology

GULF SOUTH RESEARCH INSTITUTE (NIH-70-2299)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Benjamin Sweet

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand white rabbit (Oryctologus cuniculi) which would be suitable for use in the production of viral vaccines (human).

Methods Employed: Fetal tissues from selected rabbits are propagated *in vitro* under optimal growth conditions. Cell strains are characterized with respect to growth potential, viral susceptibility, chromosomal consitution and freedom from adventitious agents.

Major Findings: During the first year of the project, fetal tissues from 3 rabbits were propagated; these included lung, heart, kidney and spleen (10 fetuses). Four strains (4 lung, 2 heart and 1 spleen) were successfully propagated beyond 10 passages. Two strains (1 heart and 1 lung) are of interest. The heart strain was normal in karyology at passage 21 with no evidence of diminished growth. The lung strain at passage 35 was normal with regard to ploidy. However, at passage 17, 23% of cells showed abnormal karyology. With this latter strain, cells taken from liquid nitrogen at passage 12 will be examined to determine whether similar changes occur again at the 17th passage.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: Those strains now showing good growth potential will be characterized further; new strains will be initiated, if necessary.

Date Contract Initiated: June 24, 1970

Current Annual Level: $119,390

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

LEDERLE LABORATORIES (NIH-69-100)

Title: Development of Sub-human Primate Diploid Cell Strains

Contractor's Project Director: Dr. Paul Vasington

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To develop diploid cell strains from fetal tissues of both the *Macaca mulatta* and *Cercopithecus aethiops* species of monkey which will be suitable for the production of viral vaccines for human use.

Methods Employed: Fetal tissues from selected pregnant monkeys will be used to initiate cell cultures which will then be characterized with respect to growth, nutrient requirements, chromosome complement, oncogenic potential, viral susceptibility, and freedom from adventitious agents.

Major Findings: A new system to screen cell cultures for oncogenic potential has been developed. Cell cultures from both species have been propagated to the point of senescence. They retain susceptibility to several viruses, remain diploid, and appear free of adventitious agents. Two cell lines are now ready for distribution to responsible investigators for independent evaluation as possible substrates for vaccine production.

Significance to DBS Program and Bio-Medical Research: The availability of a well-characterized cell system having genetic stability will allow exploration of the potential for vaccine production in cells other than primary cell cultures or human cell strains.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1972.

Date Contract Initiated: Approximately May 1, 1969

Current Annual Level: $155,000

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

Section on Immunology

TULANE UNIVERSITY (NIH-70-2300)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Ambhang D. Felsenfeld

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand white rabbit (Oryctologus cuniculi) which would be suitable for use in the production of viral vaccines (human).

Methods Employed: Fetal tissues from selected rabbits are propagated in vitro under optimal growth conditions. Cell strains are characterized with respect to growth potential, viral susceptibility, chromosomal constitution and freedom from adventitious agents.

Major Findings: During the first year of the contract, 28 cell lines were established from the fetuses of 6 dams. These cell lines are in passages 8 to 30 at the present time. All strains have been stored in liquid nitrogen and can be successfully thawed. Preliminary chromosomal studies indicate that the majority of low passage cultures are 70-80% diploid. Contamination rate (bacterial and fungae) was less than 5%.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: This project will continue; cell strains will be further characterized and new ones initiated, if necessary.

Date Contract Initiated: June 25, 1971

Current Annual Level: $57,773

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

Section on Immunology

WYETH LABORATORIES (NIH-70-2298)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Howard Tint

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand rabbit (Oryctolagus cuniculi) which would be suitable for use in the production of viral vaccines (human).

Methods Employed: Fetal tissues from selected rabbits are propagated *in vitro* under optimal growth conditions. Cell strains are characterized with respect to growth potential, virus susceptibility, chromosomal constitution and freedom from adventitious agents.

Major Findings: Cell strains have been derived from three pregnancy-timed rabbits obtained from the Jackson Memorial Laboratory, Bar Harbor. Tissue was taken from lung, heart, kidney, skin, spleen and corneal lens and prepared by standard trypsinization techniques. Cultures derived from liver, heart, spleen and kidney grew slowly and died after 15-20 passages. Favorable growth has been obtained with skin, lung and cornea; these strains are being characterized.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: The program of this laboratory will now be directed completely to karyologic studies in cooperation with the group at Tulane. Efforts will also be made to determine the normal karyology of rabbits using primary tissues and peripheral leukocytes.

Date Contract Initiated: June 23, 1970

Current Annual Level: $118,247

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

HAZLETON LABORATORIES, INC. (NIH-71-2040)

Title: Housing and Special Care of Chimpanzees

Contractor's Project Director: Dan W. Dalgard, D.V.M.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Francis V. Chisari, M.D., LP

Objectives: This contract is to provide facilities and technical support for a program designed to evaluate chimpanzees as an animal model for studies of hepatitis-associated antigen (HAA).

Methods Employed: Animals are pre-screened for HAA and anti-HAA prior to purchase and antibody-free animals are selected for inoculation studies. Baseline chemistries and open liver biopsies are done before inoculation; biopsies will be repeated to obtain presumably infected tissue for morphologic, fluorescent antibody and tissue culture studies.

Major Findings: To date two chimpanzees have been inoculated with the NIH-6 icterogenic plasma pool. One animal has been followed for 3 months post-inoculation and has not developed antigen, antibody or enzyme elevations. The second animal has only been on test for one week at this time.

Significance to DBS Program and Bio-Medical Research: The development of an animal model for HAA positive hepatitis would be a valuable asset in future studies of active and/or passive immunization against this disease.

Proposed Course of Project: This project will be continued.

Date Contract Initiated: November 6, 1970

Current Annual Level: $25,868

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

STATE OF NEW JERSEY DEPARTMENT OF HEALTH (NIH-70-2026)

Title: Epidemiologic Study of Transfusion-Associated Hepatitis

Contractor's Project Director: Martin Goldfield, M.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Michael R. Peterson, M.D., LVI

Objectives: To study the significance of positive tests for hepatitis-associated (Australia or SH) antigen (HAA) in blood donors of New Jersey, as related to the sensitivity and specificity of the available tests, in detecting those units of blood associated with hepatitis in recipients.

Methods Employed: HAA testing is done by complement fixation (CF) and counterelectrophoresis (CEP). Prospective and retrospective evaluation of antigen positive and antigen negative units of blood is being completed. As of December, 1970, over 200,000 donor units had been tested.

Major Findings: In the 1st year of this contract the CF procedure was found to be significantly more sensitive than agar gel diffusion for donor screening for HAA. During the 2nd year screening has been done by the CF and CEP methods; the latter detects approximately 92% of all positives detected by the former. In commercial blood banks a large proportion of HAA positive units came from multiple donations by a much smaller nucleus of donors who are carriers of persistent HAA.

Prospective studies of recipients has revealed no evidence of hepatic derangement or Au antigen infection in 31% of the recipients of Au positive pints in contrast with 73% of recipients of a control negative unit. Of 26 recipients who developed overt, recognized hepatitis, 7 (26%) succumbed to the disease. Evidence of chronic enzyme elevations has been found in 15% of the recipients of positive blood and 8% of the controls. Prolonged follow-up of prospectively studied recipients has been instituted to determine the significance of the persistent abnormalities in liver function tests. During the second year of the contract all donor units have been screened prior to administration and, so far as possible, no HAA positive units have been administered. The impact of eliminating HAA positive units is being evaluated. From the prospective studies a reduction of overt hepatitis risk of at least 20-25% is anticipated.

Significance to DBS Program and Bio-Medical Research: Work on the relationship of HAA to post-transfusion hepatitis and on the available tests for HAA detection is directly related to the program of the Division.

Proposed Course of the Project: This project will be continued.

Date Contract Initiated: October 1, 1969

Current Annual Level: $293,700

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

UNIVERSITY OF NORTH CAROLINA, SCHOOL OF MEDICINE (NIH-69-2197)

Title: To Investigate the Application of Large Volume Zonal Centrifugation, Purification, Analysis, and Testing of Biological Material

Contractor's Project Director: D. Gordon Sharp, Ph.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Ruth C. Dunlap, Ph.D., LVI

Objectives: (1) To develop efficient methods for concentrating and partially purifying the hepatitis associated antigen. (2) To determine biophysical characteristics of smallpox vaccine viruses in the zonal ultracentrifuge.

Methods Employed: Centrifugation in the BXIV zonal rotor, electron microscopy and standard serologic and virologic methods for quantitating hepatitis-associated antigen (HAA) and vaccinia virus.

Major Findings: (1) Hepatitis-associated antigen (HAA) from 400 ml volumes of serum has been concentrated in gradients of different compositions and configurations using the BXIV zonal rotor. Good concentrations were obtained in isopycnic (24 hr., 30,000 r.p.m.) bands in both sucrose and CsCl, also in much shorter runs (6 hr.) using a less dense step zone with a dense cushion layer. Ultimate band width (ultimate concentration) seems to be dependent upon the steepness of the gradient in the cushion layer in step gradient runs. Velocity spectrum analyses are in progress to examine the physical homogeneity of HAA. Large amounts of purified HAA have been made available for use in passive hemagglutination and radio-immunoassay procedures. Purification of antigenically distinguishable HAA sub-types for animal immunization and antigenic analysis is in progress. (2) Sedimentation velocity (SV) spectra have revealed marked individuality among several smallpox vaccines as well as a wide range of physical heterogenicity among the virions in all of them. Marked differences in SV spectra have been seen on passage of these vaccinia strains to chorsoallantoic membranes and for primary chick embryo fibroblast tissue cultures.

Significance to DBS Program and Bio-Medical Research: Work on the HAA, which is closely related to viral hepatitis, and on smallpox vaccine viruses is directly related to the program of the Division.

Proposed Course of Project: This project will be continued.

Date Contract Initiated: July 1, 1969

Current Annual Level: $49,821

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

Allergenic Products Section

OHIO STATE UNIVERSITY (NIH-69-65)

Title: Identification, Isolation, and Culture of Mites from House Dust

Contractor's Project Director: G. W. Wharton, Ph. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): E. B. Seligmann, Jr., Ph. D.

Objectives: (1) To examine house dust samples from various parts of the United States for their content of mites and other insects. (2) To determine the genus and species of the mites. (3) To isolate each species of mite, to produce pure cultures of each, and grow gram quantities of each. (4) To study the nutritional and physical requirements for optimal growth of each species.

Methods Employed: (1) Various support substances such as dust linters and nutrients such as dog food are used to propagate mites. (2) Investigate the influence of environmental and nutritional factors on mite growth in order to permit large scale production of several species of mites, especially those of the genus *Dermatophagoides*. (3) Determine whether it is possible to produce axenic cultures of mites or cultures grown in media low in antigenicity.

Major Findings: (1) House dust samples collected from widely different areas of the U.S. (many of which were supplied to the contractor by DBS) were shown to contain four species of mites of the genus *Dermatophagoides*. The most common species was *D. farinae* and has been called the North American house dust mite. This material has been published in *Science*. (2) Several different culture techniques have been studied and found to be useful in producing gram quantities of mites; the ingredients have been supplied to DBS by the contractor.

Significance to DBS and Bio-Medical Research: It has been suggested that the allergenic activity of dust is due to its content of mites. Therefore, the finding of mites in these dust samples is consistent with the concept. Further work will be required to determine whether or not mites in house dust can account for the allergy of individuals to dust and their skin reaction to house dust allergenic extract.

Proposed Course of Project: To continue until objectives are reached.

Date Contract Initiated: November 26, 1968

Current Annual Level: $20,000

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

Section on Immunology

YALE UNIVERSITY (PH-43-67-1463)

Title: A Statistical Approach to Human Karyotypy

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP

Objectives: The purposes of this contract are to determine the precise definition of the chromosome constitution of man and to seek the solution of certain problems relating to the chromosomal stability of cultured human cells.

Methods Employed: Leukocyte cultures were prepared from the cord blood of 4500 human newborns and approximately 1000 of their mothers. Slides of metaphase chromosomes were prepared and photographed. Idiograms were made from the photomicrographs and the chromosome arm length measurements were determined. High speed fully automated and semi-automated techniques of chromosome measurement have been utilized on a production basis. In addition, WI-38 cell cultures have been studied in detail in order to determine their Karyotype at low, intermediate and high passage levels.

Major Findings: The WI-38 cell analysis of 4 complete experiments was completed. Generally, the data are in agreement with those published earlier by other groups. Statistical analysis revealed that cultures should be rejected if they produce values for: polyploidy - in excess of 2.4%; aneuploidy - 2.6%, breaks and gaps - 2.8% and abnormal Karyotype - 13.3%. The optimal number of cells to be examined for each parameter is as follows: polyploidy - 300, aneuploidy - 100, breaks and gaps - 100 and abnormal Karyotype - 30.

Sufficient progress towards the major objectives of this work has now been made to report that two systems for chromosome analysis, one automated (FIDAC) and one semi-automated (X,Y digitizer), are now developed to the point of being available for routine utilization. These systems are capable of detecting small variations in the human karyotype reliably and accurately, and are applicable both to the study of chromosomal variation in tissue culture lines and human populations. Norms have been establihsed from randomly selected newborns to which varient populations can be compared. Two new staining techniques have been reported in the last year which have improved the resolution and significance of cytogenetic analysis dramatically. These allow precise identification of each of the 23 pairs of chromosomes as well as characterization of specific areas of each chromosome by type of chromatin. Both techniques have been integrated into the study where possible, since they provide a better basis for characterizing chromosomal variation. It is felt to be extremely important, however, to complete the clinical and tissue culture studies with these techniques in order to harvest the full scientific potential from the study, and to adapt the quantitative techniques described above to these new staining techniques. Although more than 20,000 newborn infants have been studied in cytogenetic surveys in the United States, Canada and Scotland, this study is the only one in which clinical data, or a precise study of minor chromosomal variation or characterization of chromosome damage (breaks and rearrangements), have been recorded and therefore, it is currently the only available resource for obtaining answers to many questions relating to the interaction of the environment and chromosomes.

Significance to DBS Program and Bio-Medical Research: This contract has two primary objectives. The first objective is to establish a reliable definition of the chromosome constitution of man and the second is the solution of problems relating to stability of continuous diploid human cell lines in vitro and the karyotypic response of these cell lines to infection with selected adventitious agents.

A precise definition of the chromosome constitution of man and a better understanding of the events associated with senescence in cultured human cells will allow the Division to make recommendations concerning the use of cultured human cells in vaccine production and may lead to new tissue culture tehhniques for evaluating the safety of biologics.

Proposed Course of Project: Work under this contract was initiated June 29, 1967. The contract was renewed in 1970 and recommendation for renewal will be considered.

Current Annual Level: $173,600

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

MELOY LABORATORIES (formerly Melpar, Inc.) (#PH-43-67-1100)

Title: Studies of Latent Infections

Contractor's Project Director: Dr. John Verna

Project Officers (DBS): Dr. Carl E. Miller
Dr. Edward Fitzgerald

Objectives: To investigate primary African green monkey kidney, canine kidney, duck embryo and a continuous line of human lung fibroblasts (WI-38 cells) for covert oncogenic and/or other latent adventitious agents.

Methods Employed: Primary cultures of the above cells have been maintained in tissue culture for a minimum of 60 days. Aliquots of the cultured cells have been inoculated subcutaneously into newborn homologous animals as well as into Syrian hamsters and BALB/c mice. The WI-38 cells were inoculated into mice and hamsters. These animals were maintained and monitored for tumors and/or other pathologic responses. Currently, all mice, hamsters, ducks and dogs have been sacrificed and reported as having no significant lesions. The monkeys will reach 24 months of age and be examined within this contract year.

Major Findings: The cells inoculated produced no evidence of oncogenic potential in the mice, hamsters, ducks or dogs.

Significance to DBS Program and Bio-medical Research: This experimental approach was designed to determine the presence or absence of covert oncogenic and/or other adventitious agents in cells used for vaccine virus production. The data obtained here should assist the Division in evaluating the relative hazards of vaccine substrates and to make recommendations relating to their use.

Proposed Course of Project: Consideration is being given to renewal of this contract through 1972 to complete the waiting period and necropsy of all of the monkeys inoculated.

Date Contract Initiated: May 24, 1967

Current Annual Level: $31,400

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

LOS ALAMOS SCIENTIFIC LABORATORIES (INTERAGENCY AGREEMENT)

Title: Monitoring Ploidy of Cell Cultures by Microfluorometry

Contractor's Project Director: Dr. M. Van Dilla

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To monitor the ploidy of cell cultures by the microfluorometric measurement of the DNA content per cell.

Methods Employed: Model non-biologic systems will be used to refine the resolution of the microfluorometry apparatus now in use, and attempts will be made to detect small subpopulations of cells in cultures provided by DBS based on their different DNA content.

Major Findings: None to date

Significance to DBS Program and Bio-Medical Research: The development of a high-speed microfluorometric method for monitoring ploidy offers the possibility of a faster and statistically more accurate technique than those conventionally used to assess the chromosomal stability of cell strains which might be considered for use in vaccine production.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1972.

Date Contract Initiated: February 2, 1970

Current Annual Level: $81,475

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1971

Section on Immunology

HUNTINGDON RESEARCH CENTER (NIH-69-59)

Title: Preparation of Mycoplasma Antigens and Antisera

Contractor's Project Director: R. del Giudice

Project Officers (DBS): Hope E. Hopps, LVI
Michael F. Barile, LBP

Objectives: (1) To prepare standardized mycoplasma antigens and antisera which will be available for use by the Laboratory of Bacterial Products and other research groups within the DBS; (2) to provide a testing service for detection of mycoplasma in biological materials being used in the Division's various programs, e.g., tissue cultures, virus and rickettsial seed pools, sera, etc.; and (3) to provide services for isolation and identification of mycoplasmas from specimens obtained from other DBS contracts concerning adventitious agents.

Methods Employed: Only purified, certified mycoplasma antigens are employed. Antisera are prepared in burros, donkeys or goats. Isolation studies are performed in broth and agar media. Identification is made on all isolates where possible.

Major Findings: Preparation of antigens, antisera and fluorescent antibody (FA) conjugates is proceeding on schedule. Fourteen antigens are in preparation; 7 of these are ready for packaging. Antisera prepared against 7 agents are now being tested as are 4 FA conjugates.

During the past 6 month period, 805 specimens were submitted to HRC for testing, an increase of 131 specimens over the number submitted during the same period last year.

Publications:

Proposal for classifying human strain navel and related mycoplasma as *mycoplasma primatum* sp. n. del Giudice, R.A., Carski, T.R., Barile, M.F., Lemcke, R.M. and Tully, J.G. (To be submitted)

Significance to DBS Program and Bio-Medical Research: The mycoplasmas constitute an important group of organisms both with immediate regard to the regulatory control of vaccines and to their overall significance in human disease.

Proposed Course of Project: The project will continue.

Date Contract Initiated: September 28, 1966; renewed annually 1967-1970.

Present Level of Funding: $108,438

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

MIDWEST RESEARCH INSTITUTE (PH43-67-679)

Title: Oncogenic Evaluation of Incomplete Freund's Adjuvant in Three Strains of Mice

Contractor's Project Director: Dr. John O. MacFarlane

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officers: Dr. Carolyn Hardegree, LBP
Dr. Carl E. Miller, OD

Objectives: To evaluate the oncogenic potential of oil adjuvants.

Methods Employed: Groups of inbred and random bred mice were injected subcutaneously with peanut oil, Arlacel A emulsifier, aluminium monosterate and antigen, singly, in mixture and in emulsion. Other groups of mice received similar treatment plus a known chemical carcinogen. Gross and microscopic pathologic examinations of the mice were made over a period of 90 weeks to determine the development and/or acceleration of tumors.

Major Findings: All mixtures or emulsions of reagents that contained aluminium monostearate were significantly oncogenic in Swiss male and female mice. Peanut oil alone and mixtures of peanut oil and Arlacel A were not oncogenic. Arlacel A sterilized by heat was less oncogenic than Arlacel A sterilized by filtration. House dust emulsions caused significant tumor induction but no significant oncogenic effects were produced by influenza vaccine emulsions. Most tumors were observed in random-bred Swiss mice; relatively few in inbred C57BL6 and Balb/C mice.

In cocarcinogenic studies all reagents and emulsions caused significant tumor enhancement in all mouse strains. The strongest promoters were mixtures of peanut oil-aluminium monostearate and peanut oil-Arlacel A-aluminium monostearate.

Significance to DBS Program and Biomedical Research: Evaluation of adjuvant preparations in animals will provide data on the safety of these materials. The results of these and other studies will provide a basis for decisions on the use and potential safety of oil adjuvants.

Proposed Course of Project: The contract has been extended to include studies of aluminium containing adjuvants. Funds have been allocated through March 31, 1972 and the contract is expected to continue into 1974.

Date Contract Initiated: February 24, 1967

Current Annual Level: $221,895

Publication: MacFarlane, J.O., Roberts, D.N., Bailey, C.A., Monley, A., Cohen, P. and Hardegree, M.C.: Oncogenic evaluation of incomplete Freund's adjuvant in three strains of mice. Federation Proceedings 29 (#2), March-April 1970.

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

Allergenic Products Section

COLUMBIA UNIVERSITY (PH-43-64-76)

Title: Biochemical Studies on Poison Ivy, Poison Oak and Sumac

Contractor's Project Director: Prof. Charles R. Dawson

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Mitchell H. Friedlaender, M.D.

Objectives: (1) Isolate the active principles of poison ivy, poison oak, and sumac and to supply these to the Project Officer for laboratory and clinical studies. (2) Prepare and characterize catechol-protein and polypeptide conjugates and to supply adequate quantities to the Project Officer for immunological studies. (3) Investigate enzymes of plant and animal origin that can oxidize or otherwise alter the pentadecylcatechols. (4) Characterize as completely as possible the components of poison oak and sumac urushiols. (5) Synthesize various substances related to pentadecylcatechol.

Methods Employed: Various isolation, synthetic, and analytical methods are employed.

Major Findings: (1) Additional quantities of the pure urushiols of poison ivy and oak have been prepared for use by DBS and for clinical studies. The method for isolation has been markedly improved. (2) The purified components of urushiol have been supplied to DBS for animal and human testing. (3) Studies are being conducted on methods of preparation and analysis of protein conjugates of pentadecylcatechol. Some of these conjugates have been sent to DBS for animal and immunological studies. (4) Various chemicals related to urushiol have been synthesized that are very poor sensitizers but are able to induce immune tolerance to pentadecylcatechol.

Significance to DBS and Bio-Medical Research: This project will provide information on a group of licensed products, Poison Ivy Extract, Poison Oak Extract, and, eventually, Poison Sumac Extract, and related products that are potentially subject to license, i.e., Cashew Nut Oil Extract. The synthetic substances being examined for the relationship of chemical structure to ability of catechols to sensitize may provide a less reactive substitute for the naturally occurring catechols for diagnosis or treatment of poison ivy, oak, or sumac sensitivity. As a result of this work, it should be possible to develop a potency assay for poison oak and ivy extracts that can be applied to products manufactured under license.

Proposed Course of Project: To continue until objectives are reached.

Date Contract Initiated: November 18, 1963

Current Annual Level: $37,629

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

Allergenic Products Section

UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL (PH-43-68-632)

Title: Determination of Potency and Efficacy of the Active Ingredient of Poison Oak Extract by Clinical Studies

Contractor's Project Director: William B. Epstein, M.D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Mitchell H. Friedlaender, M.D.

Objectives: To determine whether a pure preparation of the active principle of poison oak can hyposensitize individuals sensitive to poison oak.

Methods Employed: The active principle was isolated from fresh leaves under Contract (PH-43-64-76) at Columbia University and was prepared for clinical use by the Pharmaceutical Development Service of the NIH. The contractor administers this material to sensitive patients orally, parenterally and topically. Skin tests prior to and during treatment are used to assess efficacy. Blood and urine samples are taken at regular intervals for laboratory examination to aid in determining whether the treatment produces any toxic effects.

Major Findings: Preliminary analysis of the results of the double-blind study of the efficacy of poison oak extract in reducing the sensitivity of sensitive human beings indicates that some desensitization was achieved. This study is progressing with more individuals.

Significance to DBS and Bio-Medical Research: It has been often stated, but not scientifically established, that the administration of poison oak extracts may reduce or eliminate the sensitivity of sensitive individuals. Since Poison Oak Extracts are subject to license, it would be of value to DBS to determine if they possess efficacy. If efficacy is established, it may be possible to establish useful dosage schedules, dosage sizes and the duration of the effect. It may also be possible to obtain information relating to the mechanism thus achieving a greater understanding of delayed contact sensitivity.

Proposed Course of Project: (1) To continue the study until it can be determined with certainty that desensitization does occur. (2) To ascertain a suitable dose and schedule of administration. (3) To determine the duration of desensitization after treatment is discontinued.

Date Contract Initiated: January 15, 1968

Current Annual Level: $22,501

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1970 through June 30, 1971

Allergenic Products Section

PENNSYLVANIA STATE UNIVERSITY (PH-43-68-1250)

Title: Study of the Biochemical Properties of Pollens and Pollen Extracts

Contractor's Project Director: Allen W. Benton, Ph. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry P. Godfrey, M.D.

Objectives: (1) To carry out various physical and biochemical analyses of pollen extracts, including tests for enzymes. (2) To study the effects of various extracting fluids and methods and time of storage on the various components of the pollen extracts. (3) The effect of viability of the pollen and its age on the components of pollen extracts.

Methods Employed: (1) Pollens will be purchased at appropriate season of the year and extracts prepared using various extracting fluids including those commonly employed for preparing extracts. (2) Extracts will be studied for total protein and for the presence of various enzymes mainly by disc electrophoresis and selective staining. Changes in composition of the extracts over various time intervals will be examined.

Major Findings: Some new extracting solutions seem to stabilize the extracts more than currently used extracting fluids. Analysis of short ragweed extracts by acrylamide gel electrophoresis has identified the antigen E band. Various enzymes are detectable in the gels, including a protease and phosphatases. The gel patterns of most extracts appear to change as the extracts age.

Significance to DBS and Bio-Medical Research: Pollen extracts are products subject to license. Although it is known that these extracts contain many components including proteins, none has been identified by its biochemical activity. This project provided a relatively simple method for identifying the proteins and ascribing enzymatic activity to certain of them. Definition of the nature of changes that occur in extracts during storage is applicable to specification for the dating period.

Proposed Course of Project: This contract terminated and was not renewed.

Date Contract Initiated: June 28, 1968

Current Annual Level: $19,000

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